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Official Organ of the
American Phytopathological Society

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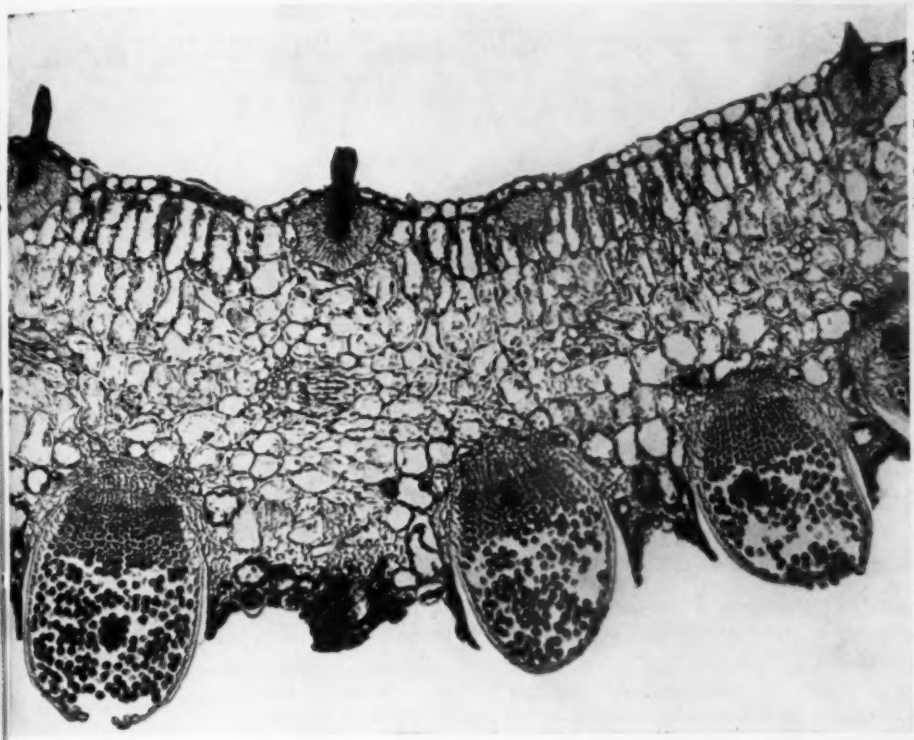

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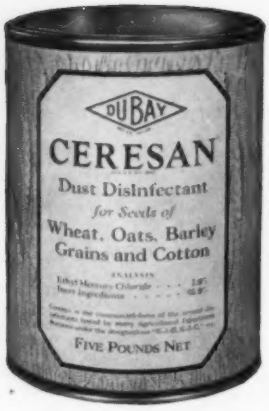

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PHYTOPATHOLOGY

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STUDIES IN THE GENUS PHYTOPHTHORA IN MYSORE

I. Heterothallic Strains of Phytophthora

M. J. Narasimhan[1]

INTRODUCTION

Sexuality in the genus Phytophthora is a subject which has recently attracted considerable attention. As is well known, in certain species such as, for example, *Phytophthora infestans* (Mont.) de Bary and *P. arecae* (Coleman) Pethyb., oospore formation is a rare occurrence in pure culture and it has not yet been observed in nature. Other species, on the contrary, such as *P. phaseoli* Thaxt., form oospores readily both in nature and in pure culture, while in a few, such as *P. erythroseptica* Pethyb., oospores seem to be the predominant reproductive body when the organisms are grown in pure culture.

The conditions under which oospores develop in *Phytophthora* spp. have not been fully determined. Clinton (5) sought first to prove that oospore production was dependent on the presence of male and female mycelial strains. With this object, he attempted to obtain single-spore cultures of *P. phaseoli* by plating melted agar containing the spore-bearing mycelium shaken through it. Since the resulting colonies ran into one another, Clinton could not be sure that he was dealing with single-spore cultures. When grown on favorable media, these individual colonies developed oospores, but whether this was due to homothallism or to the mixing of mycelia from two colonies he was not sure. Clinton (6) was able to obtain oospores by growing *P. infestans* and *P. phaseoli* together. Gadd (9) obtained a number of strains of *P. faberi* Maub. occurring in Ceylon on various plants such as cacao (pods), papaya (fruit and stem), Hevea (pods), *Artocarpus incisa* L. (fruit), *Dendrobium MacCarthiae* Thw., and *Odontadenia speciosa* Benth. All the cultures were started from single sporangia. All possible combinations of two strains were grown on agar slants. In general, oospores were produced when any of the first three strains (cacao group) were grown

[1] The writer wishes to acknowledge his indebtedness to Dr. Leslie C. Coleman, Director of Agriculture in Mysore, for the encouragement and helpful suggestions given in the course of the investigation.

with any of the other four (rubber group). Any two strains of the same group did not form oospores when grown together. This separation into two groups showed, however, some exceptions. According to Gadd, the Dendrobium strain never formed oospores with the two papaya strains, nor did the bread fruit strain with that obtained from papaya fruit.

Lester-Smith (10), working along similar lines, obtained oospores in cultures of cacao, West Indian cocoanut, and cotton strains, when each was grown with *P. parasitica* Dastur. Ashby (1) found that large oospores were formed when *P. cinnamoni* Rands was grown with *P. parasitica* and with strains of *P. cryptogaea* Pethyb. & Laf. Recently, Ashby (2), working with a large number of strains of *P. palmivora* Butl. (*P. faberi* Maubl.), adopted Gadd's grouping, viz, "cacao" and "rubber" strains. Nine strains of the cacao group, when grown in paired cultures with fourteen strains of the rubber group, were found to produce oospores, the exceptional case being that the Citrus strain from the Philippines, when paired with the cocoanut strain from India, did not produce any oospores, although these strains belonged to two different groups.

The present investigation was originally undertaken with the object of ascertaining what relationships, if any, exist between the various forms which the author has been able to collect in Mysore. During the course of this investigation interesting observations have been made on the sexuality of these forms, the preliminary results of which form the subject of this paper.

PHYTOPHTHORA STRAINS OCCURRING IN MYSORE

A brief report of the various Phytophthora strains occurring in the area where the ravages of *P. arecae* on arecanut are rampant has already been given by the writer (12). A short description of these strains and the symptoms produced by them follows.

1. *Phytophthora on Santalum album* L. Shedding of the leaves, which show brown patches, is a prominent feature of the disease produced by this fungus. The tender shoots, flowers, and fruits are all affected. Sporangia: 41 $\mu \times 25.9\ \mu$. Sexual bodies not found in nature nor in cultures.

2. *Phytophthora on Loranthus longiflorus* Desr. Parasitic on *Mangifera indica* L., *Eugenia jambolana* Lam., etc. This is also of very wide occurrence. Affected leaves turn rusty brown. Sporangia: $42.5\ \mu \times 32.8\ \mu$. No oospores found either in nature or in cultures.

3. *Phytophthora on Jatropha curcas* L. The disease appears as brown patches on the fruit which later turns black. This is the commonest form in the area as these plants serve as hedges for paddy fields. Sporangia: $37.8\ \mu \times 26.7\ \mu$. Chlamydospores ($28-29\ \mu$) are found in large numbers on

the fruits and in cultures. In oat-agar cultures the aerial mycelium practically fills the tube. The fungus answers to the description of *P. jatrophae* Jens. (*P. faberi* type.) No oospores found in nature nor in cultures.

4. *Phytophthora on Bryophyllum calycinum* Salisb. The disease appears as round patches on the leaves, often spreading in concentric rings Sporangia: 42.5 μ × 26.2 μ. Oospores occur both on fallen rotting leaves and in cultures.

5. *Phytophthora on Artocarpus integrifolia* L. Almost every tree in the area is affected by this disease which causes the shedding of leaves and rotting of the tender inflorescences. Sporangia small, round. Oospores found in abundance on the affected inflorescences. In oat-agar cultures the aerial mycelium is not at all prominent, only oospores and no sporangia being formed. Sporangia are, however, formed if the fungus is grown on sterilized flies in distilled water, as in the case of *P. erythroseptica* Pethyb.

6. *Phytophthora on Colocasia antiquorum* Schott. Probably *P. colocasiae* Rac. Oospores not found in nature and rarely in oat-agar cultures.

7. *Phytophthora on Ficus hispida* L. (*Wild Fig*). This strain is not of very wide occurrence. Only the fruits are affected and these drop. Sporangia ellipsoid, with prominent crown-like papillae. Oospores found in large numbers on the fruits and in cultures.

OOSPORE PRODUCTION IN THE ARECA, SANTALUM, LORANTHUS, AND JATROPHA STRAINS

These first four strains of Phytophthora, viz, *P. arecae* and the Santalum, Loranthus, and *Jatropha curcas* strains, form oospores rarely or not at all in nature or in culture and were selected for further study. Neither Coleman (7) nor the writer has ever observed oospores of *P. arecae* in nature or in oat-agar cultures. They were obtained by Coleman in a set of cultures, wherein fresh aseptically-taken arecanuts were placed in Roux tubes and inoculated with mycelium of *P. arecae*. It is not unlikely that material from these particular cultures was sent to Rosenbaum (13) who reported the presence of oospores in his cultures of *P. arecae*. A possible explanation for this phenomenon is indicated in the course of this paper. The writer has examined many affected arecanuts and oat-agar cultures but has never observed oospores at any time. For the present and pending further work, *P. arecae* is taken as a nonoospore-forming strain. Preliminary trials in which the mycelia of *P. arecae* and the Santalum Phytophthora were grown together on oat-agar were made with the result that oospores formed where the mycelia met. With the idea of making a careful study of this phenomenon, single-spore cultures of the four Phytophthora strains were made.

Malt-agar tubes inoculated with zoospore suspensions were poured into plates and the resulting growths from single spores were picked with the aid of a binocular microscope. Zoospores for these cultures were obtained by placing in sterilized water bits of mycelium from different tube cultures so as to ensure a fairly representative sample. Twenty-five of such single-spore cultures of each of the four Phytophthora strains under consideration were started in oat-agar tubes. It seemed certain that single-zoospore cultures formed a surer basis for work than the single-sporangium cultures of other workers, as the sporangium may contain a number of zoospores with possibly different genetic constitution.

All possible combinations of two of these various strains were grown together. At first the two bits of mycelium from single-spore cultures were grown in roll-tube cultures and examined under the microscope. Although the danger of contamination is minimized by this method, it has the disadvantage that the thin film of the medium dries up after a time. Later cultures were all made in small Petri dishes on oat-agar or French-bean agar.

Single-spore cultures of the Areca strain when grown with those of the Santalum strain always produced oospores. If a single-spore culture of *P. arecae* and one of the Santalum strain are sown an inch apart on French-bean agar, the beginning of oospore formation may be observed on the third day at the junction of the two mycelia, while on the fourth or fifth day, a brownish line indicates the area of oospore production (Fig. 1, A). It is interesting to compare this and subsequent figures with those of Blakeslee (3) for plus and minus strains of Mucor and those of Claussen (4) for the heterothallic strains of *Pericystis alvei* A. D. Betts, which causes a disease of bees known as "Kalkbrut." Under similar conditions, oospore formation, accompanied by the development of a brown line, was observed when

TABLE 1.—*Oospore-formation reaction resulting from joint culture of differing strains of Phytophthora on oat-meal and French-bean agar*

Strains grown together		Oospore formation	Oospore size
Areca	13[2] × S 11	+	30–31 μ
"	13 × L 11	–	
"	13 × J 12	+	26–27 μ
"	13 × A 11	–	
Santalum	11 × L 11	+	30–31 μ
"	11 × J 12	–	
"	11 × S 14	–	
Loranthus	11 × J 12	+	29–30 μ
"	11 × L 14	–	
Jatropha	6 × J 12	–	

[2] Numbers indicate single-spore strains.

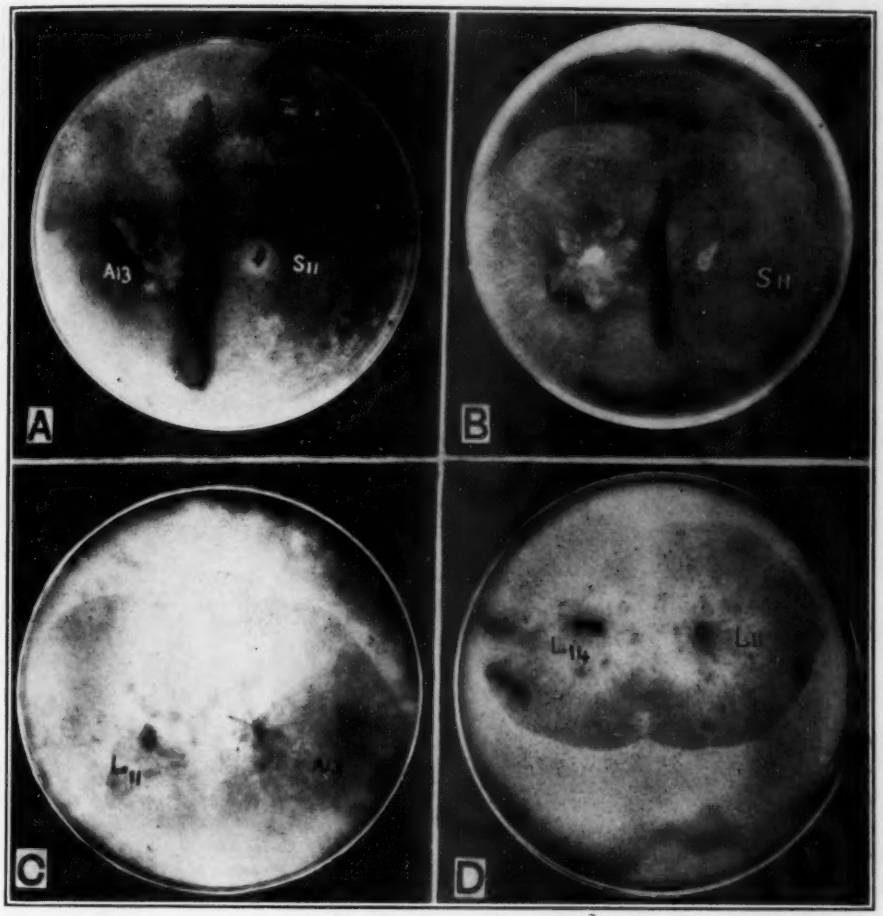

FIG. 1, A. Paired single-spore culture of the Areca (A. 13) and Santalum (S. 11) strains. Dark line in the middle indicates area of oospore formation. Photograph taken nine days after starting culture. B. Paired single-spore culture of Loranthus (L. 11) and Santalum (S. 11) strains, showing dark line at the juncture of the two mycelia Photograph taken five days after starting culture. C. Paired single-spore culture, six days old, of the Areca (A. 13) and the Loranthus (L. 11) strains. No formation of dark line. D. Paired single-spore culture of two Loranthus strains. No formation of dark line. All photographs, except B, taken with illumination from below.

single-spore cultures of the Loranthus and Santalum strains (Fig. 1, B), the Loranthus and Jatropha strains (Fig. 2, B), and the Areca and Jatropha strains (Fig. 2, C) were grown together.

In no case was oospore formation observed when single-spore cultures of the Areca and Loranthus strains (Fig. 1, C) or the Santalum and Jatropha strains (Fig. 2, A) were grown together.

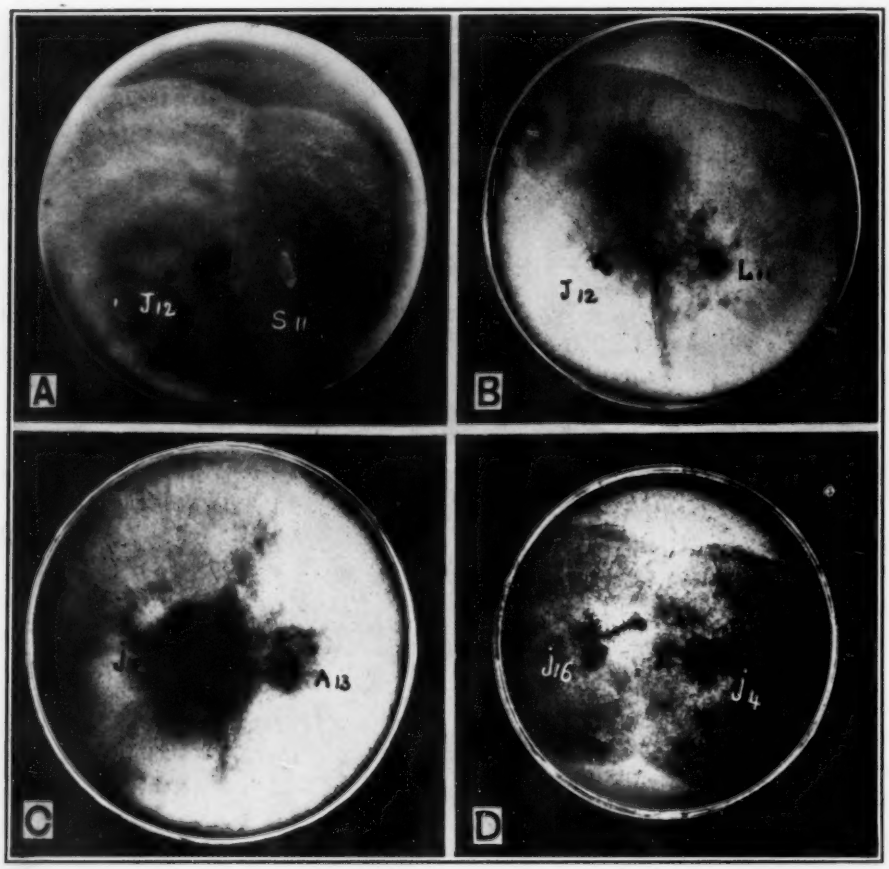

FIG. 2, A. Paired single-spore culture of the Santalum (S. 11) and Jatropha (J. 12) strains of Phytophthora. Culture six days old. No formation of dark line. B. Paired single-spore culture, five days old, of the Loranthus (L. 11) and Jatropha (J. 12) strains of Phytophthora, showing dark line. C. Paired single-spore cultures of the Areca and Jatropha strains of Phytophthora, showing dark line in the middle. D. Two paired single-spore Jatropha strains. Substage illumination for all photographs except A.

PROOF THAT ARECA STRAIN PRODUCES ANTHERIDIA; SANTALUM STRAIN, OOGONIA

In studying the details of the oospores, staining with Sudan III according to Strasburger's method was found to give excellent results. When mounted in glycerine gelatine, the oospores retained the stain for about two months. Löhnis (11) pointed out that Sudan III could be used as a differentiating stain for the mycelium of Phytophthora in tissues, while other fungi do not take this stain. The oospores were always of the amphigynous

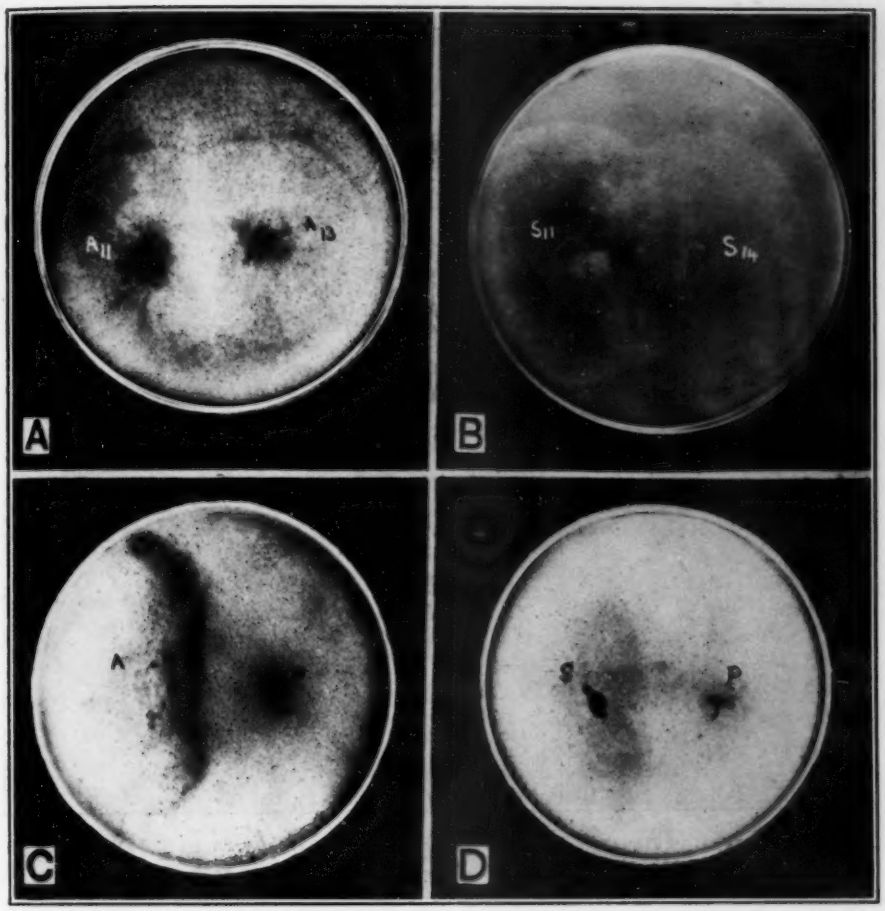

FIG. 3, A. Culture of two single-spore strains of P. arecae. B. Culture of two single-spore Santalum strains. No formation of dark line in A and B. C. Paired single-spore culture of the Areca and parasitica strains. Formation of dark line is prominent. Culture six days old. D. Paired single-spore culture of the Santalum and parasitica strains. No formation of dark line. Substage illumination for photographs except B.

type, the antheridia and oogonia being borne on different thalli. Since the oospores are formed at a place where the two thalli interlace, forming a network, great difficulty was experienced in determining which strain bears the antheridium and which the oogonium. This difficulty was partly overcome by checking the rapid advance of the two colonies by the following method:

Two strips of mica with tiny holes were so placed vertically at the bottom of the Petri dish as to intervene between the two spore sowings. It was found that the mycelium which grew through the holes in this barrier was

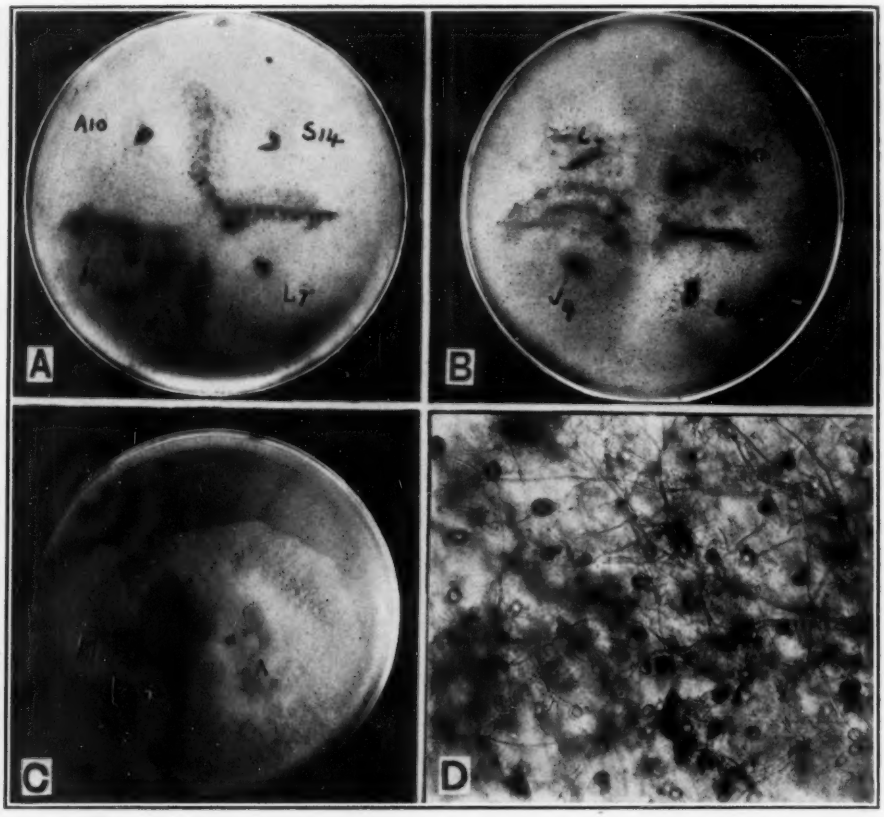

FIG. 4, A. Single-spore cultures of the Areca (A. 10), Santalum (S. 14), Loranthus (L. 7), and Jatropha (J. 4) strains so placed that a dark line formed at the place of union of each pair. B. Culture of the same four strains, arranged differently to show the formation of dark bands between strains of opposite sex only. No formation of dark line, either between the two male strains (above) or the two female strains (below). C. Paired culture of single-spore strain of *P. arecae* and *P. meadii*. Formation of dark patch in the middle can be seen. D. A portion of the dark line shown in figure 1, A, low-power magnification, showing numerous oospores. Photographs illuminated from below except in C.

sparse enough to facilitate observation. When the Areca and Santalum strains were thus grown together, it was observed that the antheridia were borne on the mycelium of the former and the oogonial incepts could be traced to the mycelium of the latter (Fig. 5, A).

Up to the present, the work done has related to four strains of Phytophthora which normally do not produce oospores either in nature or in cultures. The exceptional case wherein oospores were reported in *P. arecae*

by Coleman (7) may perhaps be explained by assuming that the Areca and Santalum strains have by some chance found their way into the culture. If we take into consideration the fact that these two strains of Phytophthora are found in close proximity in nature, breaking out almost at the same time, such a possibility is not excluded. How far such a mingling of these two strains occurs in nature is yet to be determined.

OOSPORE PRODUCTION IN PHYTOPHTHORA PARASITICA AND P. MEADII

Through the kindness of Mr. W. McRae, Imperial Mycologist, Research Institute, Pusa, cultures of *P. parasitica* and *P. meadii* were obtained. Though oospores had been reported previously in these two Phytophthoras by Dastur (8) and McRae, respectively, no oospores developed either in the tube cultures originally received or in our subcultures. Probably these cultures had lost the power of forming oospores, as has been reported in other species of Phytophthora by various workers.

TABLE 2.—*Showing results obtained by pairing P. parasitica and P. meadii with the Areca, Loranthus, Santalum, and Jatropha strains*

Strains grown together			Oospore formation
Areca	×	parasitica	+
Loranthus	×	parasitica	+
Santalum	×	parasitica	−
Jatropha	×	parasitica	−
Areca	×	meadii	+
Loranthus	×	meadii	+
Santalum	×	meadii	−
Jatropha	×	meadii	−

It therefore appears that *Phytophthora parasitica* and *P. meadii* resemble the Santalum strain in that they form oospores when paired with either the Areca or the Loranthus strain.

DISCUSSION OF RESULTS

The results given above seem to indicate quite clearly that heterothallism exists in some species of Phytophthora. Before attempting to evaluate the results obtained, it is necessary to consider whether, in an oospore-forming or apparently homothallic species, two sexual strains, male and female, actually exist. Clinton (5) attempted to separate two such strains in the case of *P. phaseoli*, grown in pure culture, but was not able to obtain definite results. Whether homothallism exists in certain species here under investigation is a question now being studied.

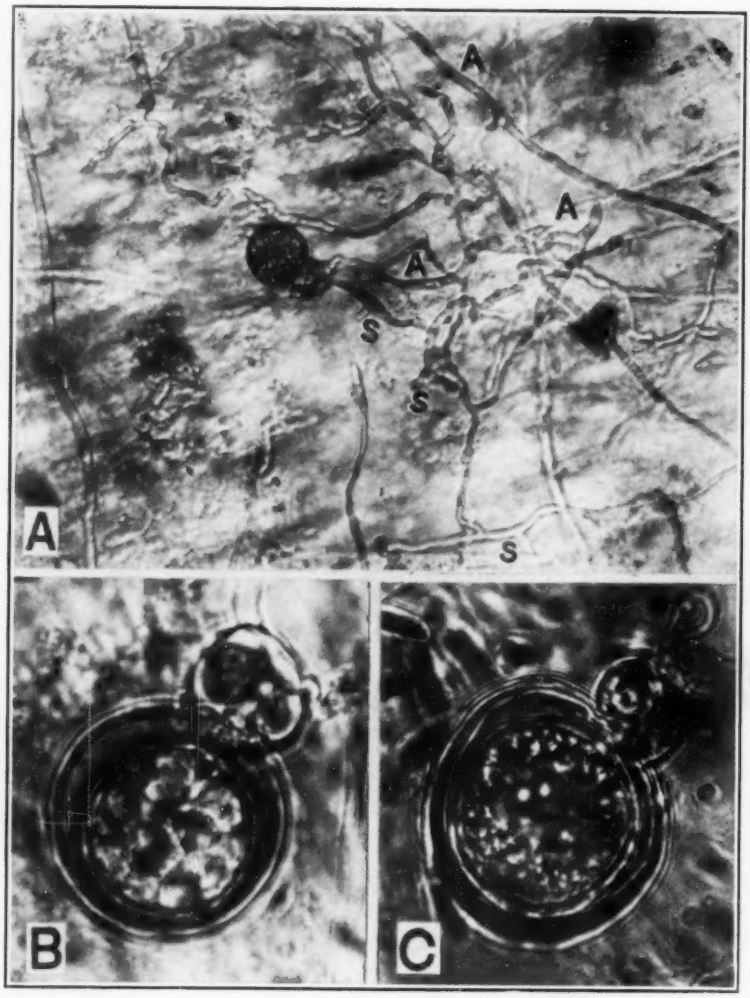

FIG. 5, A. Photomicrograph showing the mycelial relationship in formation of an oospore in a paired culture of the Areca and Santalum strains. (A) Mycelium of the Areca strain attached to the antheridium and (S), the mycelium of the Santalum strain attached to the oogonial incept. Photograph taken directly from a Petri-dish culture. B. Photomicrograph of an oospore formed in a paired culture of the Areca and Santalum strains × 900. C. Photomicrograph of an oospore formed in a paired culture of the Areca and Jatropha strains × 900.

The behavior of the three strains, Areca, Loranthus, and Santalum, can be explained if we suppose that the first two are male and the third, female. Oospores always formed when the Santalum strain was grown with either of the other two, but under no conditions were they formed when the Areca

and Loranthus (male) strains were grown together. As stated above, the mycelium of the Areca strain has been shown to bear the antheridia when single-spore cultures of this strain are grown along with the Santalum strain.

Lester-Smith (10), working with cultures of various strains of *P. palmivora* paired with *P. parasitica*, held the view that a normal strain of *P. palmivora* was potentially bisexual and that, in mixed cultures, "a low rate of metabolism characterized by low water content and a different ratio of food materials" induced by the presence of a competing organism, resulted in the development of oospores. This view does not explain why oospores are produced only when certain strains are paired.

While it is probable that some change in the media takes place where two oospore-forming strains meet, as evidenced by the brown coloration, it cannot be definitely said whether this is due to some sort of staling substance or to the thick brown walls of the oospores. The results obtained, however, seem to show clearly that the formation of oospore is due not to any effect of growth on the medium but to the union of two strains of opposite sex.

That the Areca and Loranthus strains are male and the Santalum strain is female is supported by the results obtained from the crosses made with *P. parasitica* and *P. meadii*, in which oospores were formed only when these species were grown with the Areca and Loranthus strains and not with the Santalum and Jatropha strains. The cultures of *P. parasitica* and *P. meadii* used in this work probably contained only female strains of mycelia, but a careful microscopic examination of the cultures, such as has been carried out in the case of the Areca-Santalum combination, will have to be made before this question can be definitely settled. These two species, though originally bisexual and able to produce oospores, had lost their power of forming them when received from Pusa by the writer. This loss of the power to produce oospores in culture was observed by Clinton (5), Rosenbaum (13), and Dastur (8). Clinton was of the opinion that in cultures of *P. infestans* which failed to show oospores in culture the male mycelium had deteriorated, since the oogonia appeared more frequently than antheridia on oat-agar.

The interesting question remains as to whether, in the cases observed, we have to do with male and female strains of the same species or whether the oospores are the hybrid product of the union of male and female strains of different species. While the question requires very much more thorough investigation than has yet been possible, it seems probable that we have had both phenomena under observation.

The Santalum and Areca strains are morphologically almost indistinguishable. Their growth in culture media is different, however, the San-

talum strain being slower in growth and exhibiting much less aerial mycelium. The oospores resulting from a union of the two strains are of the same dimensions (30–31 μ) as those observed by Coleman (23–36 μ) and Rosenbaum (29.45 μ to 31.49 μ) for *P. arecae*.

If, as seems highly probable, either the male or female strain may disappear during culture on artificial media, leaving only the other, it would seem to be not too violent a stretch of the imagination to picture a similar occurrence on natural hosts so that, for example, we might have the male strain growing normally on Areca and the female strain on Santalum. These two host plants are found in sufficiently close proximity to allow for the occasional transfer of zoospores of one strain to the other host plant and mingling of mycelium in this host, thus accounting for the occasional production of oospores in the field and in culture, as noted by both Coleman and Rosenbaum. Experiments to test this possibility are at present under way.

In the case of oospore formation induced by the union of *P. arecae* with *P. parasitica*, it would appear more probable that we have to do with hybridization. The oospores in this case appear to be more or less intermediate in size (24–25 μ) between the oospores of *P. parasitica* and *P. arecae* as given in the literature. The further investigation of this interesting possibility through the germination of oospores and the study of subsequent generations will be taken up immediately.

The observations that have been presented in this paper are admittedly of a preliminary character. It has, however, been definitely established that the formation of oospores in paired cultures of two separate strains results from a union of the two strains which can be distinguished as male and female. In these cases oospore formation commences in three or four days. It has further been shown that where two male strains (Areca and Loranthus) are grown together no oospores are formed. Similarly, where two female strains (Santalum and Jatropha) are grown together no oospores are formed and in this case, we are apparently dealing with female strains of two different species. All the evidence so far collected supports Gadd's view that we are dealing with heterothallic forms of which the male and female strains have become more or less definitely isolated on different host plants. The possibility that absence of oospore formation in nature or loss of it in culture, so commonly met with in this genus, is due to the loss of one or other of the two sex strains and not to any external conditions merits serious investigation.

SUMMARY

In addition to *Phytophthora arecae*, seven strains of Phytophthora were collected in Mysore, occurring on *Santalum album* L., *Loranthus longiflorus*

Desv., *Jatropha curcas* L., *Bryophyllum calycinum* Salisb., *Artocarpus integrifolia* L., *Colocasia antiquorum* Schott, and *Ficus hispida* L.

Single-spore cultures of four of these strains, viz, those on Areca, Santalum, Loranthus, and Jatropha, were obtained and grown in paired cultures.

When single-spore cultures of the Areca or Loranthus strain were grown with any of those of the Santalum or Jatropha strain, oospores were formed at the juncture of the two mycelia, the area being indicated by the formation of a brown line.

Oospores did not develop when Areca and Loranthus strains or Santalum and Jatropha strains were grown together, nor was there any formation of the brown line.

When the Areca and Santalum strains were paired, the antheridia were traced microscopically to the mycelium of the Areca strain, the oogonia to that of the Santalum strain.

The view is held that oospore formation is the result of the fusion of heterothallic strains, the Areca and Loranthus strains having the male mycelia and the Santalum and Jatropha strains the female mycelia.

Cultures of *P. parasitica* and *P. meadii*, which had lost their capacity for oospore formation when grown with a male strain, such as *P. arecae*, readily formed oospores, but did not, when grown with a female strain, such as the one on Santalum. It is possible that the male strain in each of the cultures had been lost or had deteriorated.

The possibility of the two sexual strains becoming isolated on different host plants in nature is indicated as an explanation for the absence of oospores in some of the Phytophthoras.

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POTATO BLACKLEG: THE SURVIVAL OF THE PATHOGENE IN THE SOIL AND SOME FACTORS INFLUENCING INFECTION[1]

J. G. LEACH[2]

Although Appel (1) in his extensive investigations of potato blackleg presented evidence indicating that the pathogene could overwinter in the soil, many investigators in later years have reached opposite conclusions. Morse (12) after nine years of investigation, stated that "observations in Maine indicate that under the climatic conditions which exist there, infected seed potatoes are the sole source of infection and distribution and that the disease does not live over winter in the soil." Pethybridge (17), in 1912, expressed the opinion that the pathogene did not overwinter in the soil in Ireland. The sporadic appearances of the disease in the field and the abundant evidence that the pathogene is transmitted in seed tubers apparently have caused many people to assume, without sufficient evidence, that the pathogene does not survive in the soil.

The first attempts made in the United States to answer this question experimentally were made by Rosenbaum and Ramsey (19), who worked in Aroostook County, Maine, and at Norfolk, Virginia. They placed tubers thoroughly rotted by the blackleg pathogene in the soil in October. The following spring they planted disinfected tubers in the rows where the diseased tubers had been placed the previous fall. These tubers grew normally and no blackleg appeared. They were also unsuccessful in their efforts to reisolate the pathogene from the decayed tubers or from the soil about them. They stated that "In no case, therefore, was the blackleg organism found to live over a winter in the soil or in the tubers remaining in the soil."

In 1919 Ramsey (18) reported the results of additional experiments made in Aroostook County, Maine. Half-pint glass jars were filled with soil and 15 cc. of a virulent culture of *Bacillus atrosepticus* van Hall were poured into the top of each jar. One set of jars was overwintered out-of-doors, buried 5 inches in the ground; another set was kept in a cool base-

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[2] Some of the work reported in this paper was done while the author was a Fellow of the International Education Board, working in the laboratories of Dr. Sydney Paine, Imperial College of Science and Technology, London, England, and Dr. C. Stapp, Biologische Reichsanstalt, Berlin-Dahlem, Germany. The author expresses to them his appreciation for the facilities provided as well as for their helpful criticism and the many courtesies shown him.

ment. In the spring "dilution plates were made from the jars and all favorable looking colonies were tested for pathogenicity upon cut slices of potato tuber." None of the cultures isolated was found to be pathogenic.

Two additional experiments were made in which healthy tubers were planted in pots and watered frequently with broth cultures of the pathogene. All plants so treated remained healthy during the growing season, and at harvest time the tubers were free of rot. The author concluded that "These two experiments tend to show that unless the seed piece is infected at planting time, there is little chance that uninjured plants will contract the disease even though the causal organism is washed about the stem and root system."

Apparently, these negative results have been accepted as conclusive by most American workers in plant pathology. This is indicated by the frequency with which it is stated in extension literature that the pathogene does not survive in the soil and that infected seed stocks are the sole source of infection.

Stapp (21) has recently published the results of his extensive investigations on potato blackleg. He has demonstrated the ability of the pathogene to survive exposure to the winter conditions in Germany, both as a pure culture on agar and in the remains of infected tubers or vines buried in the soil. He did not, however, attempt to isolate the pathogene from the soil.

During the past five years considerable evidence has been obtained by the writer that the blackleg pathogene can and does survive in the soil under the severe winter weather conditions which characterize Minnesota. He has also obtained evidence to show that the negative results obtained by Rosenbaum and Ramsey (19) and Ramsey (18) do not in any way prove that the organism does not survive in the soil.

In 1926 (10) the writer described his efforts to produce the disease by seed-piece inoculation. A large number of tubers were artificially inoculated with a virulent culture of the pathogene and were incubated in a moist chamber until a considerable portion of each tuber had decayed. When these partly rotted tubers were planted in the soil under conditions favorable for germination, the decayed portions were walled off by a layer of wound cork and the disease developed no further. These experiments have been continued for the past three years with similar results. A few blackleg plants have appeared in the plots planted with such inoculated tubers, but the percentage has always been extremely small. In experiments extending over five years and including over 2,000 seed pieces, the increase in the amount of blackleg developing from inoculated tubers as compared with that developing in the check plots was less than one per cent.

When we consider the difficulty of producing the disease by the methods used in these experiments, it is obvious that the failure of the disease to

develop in the experiments of Rosenbaum and Ramsey (19) and Ramsey (18) does not prove that the bacteria are unable to survive in the soil. On the other hand, it shows that the bacteria, even if present in great abundance, are unable to infect an uninjured seed piece. The writer has repeated the experiments of Rosenbaum and Ramsey and has obtained similar negative results, but he is forced to interpret them differently.

Rosenbaum and Ramsey (19) and Ramsey (18) attempted to isolate the bacteria in the spring from soil heavily inoculated the previous fall but were unable to do so. The writer has repeated these experiments also, but, by using a suitable technique, it has been possible to isolate the blackleg pathogene not only from such inoculated soil but also from several samples of soil taken at random from potato fields in early spring before the appearance of the disease. Rosenbaum and Ramsey evidently poured dilution plates from the soil and attempted to isolate the organism by picking out "favorable looking colonies." When one considers the great number of bacteria which inhabit most soils and the similarity of their appearance on agar, it is not surprising that they did not succeed in reisolating the pathogene. Realizing this difficulty, the writer proceeded in the following manner. About one gram of the soil to be tested was thoroughly mixed with about 10 cc. of sterile beef-extract broth. This suspension was used for inoculating several surface-sterilized tubers by the "hole and plug" method previously described (10). After incubation in a moist chamber for four or five days a considerable amount of decay had developed. This decay was quite unlike that resulting from a pure culture of the blackleg pathogene and advanced much more slowly. It was usually accompanied by a foul odor and frequently gas production was evident. Most of the decayed tissue was next removed with a scalpel and a small portion at the margin nearest the sound tissue was removed with a sterile needle and used for inoculating a second lot of tubers or tuber slices. When these had started to decay, a third lot were inoculated in the same manner. Usually the virulence of the inoculum increased with each successive inoculation, and after three or four transfers the decay was quite typical of blackleg. When a small amount of freshly decayed tissue from the fourth or fifth inoculation was used for pouring separation plates, little difficulty was experienced in isolating a vigorously pathogenic culture similar in all respects to the culture used for inoculating the soil.

Patel (15), by using crystal violet bile agar as a selective medium, has recently demonstrated that the organism may survive the winter in sterilized and nonsterilized soil in Iowa. In this connection should be mentioned also the experiments of Kotila and Coons (9). They thoroughly inoculated nonsterilized soil in wooden pails in the fall and allowed part to overwinter in

a greenhouse and part out-of-doors. In the spring "instead of attempting the almost futile task of isolating the organism from the soil by the plating method, raw tuber slices were inoculated with small portions of the soil, thus allowing the tuber slices, the natural host, to do the selecting. One hundred and eight tuber slices were inoculated. . . . After five days, rotting was evident on a few slices and after a still longer incubation period, it was observed that ninety of the possible 108 tuber slices were rotting. It should be recorded that from the soils which were used as inoculum in this experiment, there were produced on the potato slices mixed cultures of bacteria and fungi, and that some of the slices showed discoloration not entirely dissimilar to the blackening which appears on tubers from blackleg. It is to be noted that a long time period elapsed before rotting began, many times greater than the regular period for blackleg bacteria to produce rot. Certain soil fungi, and associated bacteria, are capable of producing discoloration and rotting of tubers and these are likely to have been responsible for the rotting in this experiment. Attempts to isolate the blackleg organism directly from the rotting slices or from plants inoculated from the discolored tuber slices resulted in failure. It is doubtful,therefore, if any of the rotting was of the *B. atrosepticus* type. The conclusion, therefore, seems justified that under the conditions of this experiment, the blackleg organism did not overwinter in the soil, either in the greenhouse or out-of-doors."

In the opinion of the present writer, the above conclusion is not entirely justified. Kotila and Coons (9) show in this same paper that when tuber slices are inoculated with a very small quantity of bacteria no decay is evident before five days, while, when large quantities are used, the decay appeared in two days. Now it is to be expected that the blackleg pathogene, after overwintering in nonsterilized soil in competition with the natural soil flora, would be reduced to relatively small numbers. But, when such material is used as inoculum, they conclude, because the resulting decay is slow in appearing, that certain soil fungi and bacteria were responsible for the decay. In view of his own experiments, the writer is quite confident that had they continued their selective inoculations further they would have been able to reisolate the pathogene with little difficulty.

Both sterilized and nonsterilized soils have been used in the writer's overwintering experiments. Periodic isolations have shown that the relative abundance of the bateria decreases so rapidly in nonsterilized soils that after a few days it is difficult to reisolate the pathogene without resort to the selective method described above. In sterilized soils the decline is much slower and the bacteria are relatively abundant after many months of exposure.

Coons and Kotila (4) isolated a bacteriophage from the soil and concluded that in all probability it played a prominent part in the decline of

B. atrosepticus and *B. carotovorus* in the soil. The writer has not worked with this bacteriophage, but it is evident that whatever factor may be responsible for the decline, it does not result in the complete elimination of the blackleg pathogene.

Many workers have pointed out that the blackleg pathogene is resistant to low temperatures. Jennison (7) exposed the organism in sterilized soil for 24 hours to a temperature varying from −28° C. to −6.7° C. without any apparent injury. In the writer's experiment the bacteria have been exposed in sterile and nonsterile soils for an entire winter, during which the temperature was as low as −25° F. and remained below 0° F. for long periods. Although the bacteria apparently were reduced in abundance, some of them were not killed and they were readily reisolated.

Many investigators have called attention to the sensitiveness of the blackleg pathogene to desiccation. Morse (12), Smith (20), and others have shown that the organism is killed in a few hours if dried on the surface of glass at room temperature and humidity. Jennison (7), on the other hand, states that it is quite resistant to drying and remains long viable on plain beef agar. Kotila and Coons (9) found that, when dried on silk threads, the organism would survive four days, while, under the same conditions, it died within a few hours if dried on the surface of cover-glasses. They refer to the work of Giltner and Langworthy (5), who have shown that many non-spore-bearing bacteria are able to survive in air-dried soils for long periods, but Kotila and Coons did not report any experiments of this type. In so far as the writer could learn, no experiments have ever been reported in which the survival of the pathogene in dry soils has been tested. It was decided, therefore, to attempt to find out how long the pathogene would survive in soils stored in atmospheres of different degrees of relative humidity. Before this experiment was started a sample of soil was found in the laboratory which had been collected from a potato field near Crookston, Minnesota, and had been kept for more than a year in a loosely covered cardboard box. The soil was thoroughly dried and had a moisture content of 1.98 per cent. By using the selective method of isolation a vigorously pathogenic culture was isolated from this soil which in all salient characters was identical with authentic cultures of the blackleg pathogene. This alone wiuld indicate that the organism is able to survive at least a year in very dry soil. However, it was decided to make additional tests under controlled conditions.

Two types of soil were used, one the soil from Crookston, Minnesota, mentioned above, and the other a rich garden loam from a garden near London, England. The pH value of the former soil was found to be approximately 7.5 and of the latter 5.8. Each sample was oven-dried and then finely pulverized. Fourteen five-gram samples of each soil were prepared

and put into small test-tubes which were then plugged with cotton. Two tubes of each type of soil were placed in each of seven large-mouth bottles, the bottoms of which were padded with glass wool. The bottles were then plugged with cotton, placed in an autoclave, and sterilized at 20 pounds pressure for three hours on three successive days. Representative samples of the soil were then tested in broth and found sterile. Each tube was then inoculated by adding one cc. of a 24-hour broth culture of the blackleg pathogene.

Solutions of sulphuric acid and distilled water were prepared according to the table given by Stevens (22) for the production of relative humidities of 0, 9, 18.5, 38.0, 60.7, 80.5, and 100 per cent. One hundred cubic centimeters of these solutions were put into their respective bottles and the bottles were then corked, sealed with paraffin, and placed in a dark cupboard at room temperature. At intervals of approximately one month the soil in each of the tubes was tested for the presence of the pathogene by inoculating a tube of sterile broth with a loopful of soil. Two or more of the cultures obtained each time were tested for pathogenicity on potato tubers. The experiment was begun October 17, 1927, and was terminated June 15, 1928. On January 2, 1928, all the tubes were removed from the bottles and wrapped tightly in several thicknesses of wrapping paper and were kept in a handbag until January 12, when they were placed in bottles over fresh sulphuric-acid solutions. Unfortunately, no provision was made for determining the rate of desiccation, but the moisture contents of the soil samples were determined at the close of the experiment. It was evident from observation that the moisture content of some of the samples did not reach equilibrium until after December 21. Although the soils were very dry at the close of the experiment, it is possible that they would have become drier had the experiment been continued longer.

In testing the viability of the organism no counts were made to determine the numbers of bacteria present. It was obvious, however, that in the drier soils the bacteria were rapidly diminishing in number. This was indicated by the increased length of time required for the clouding of the broth and by the fact that on April 12 and June 15 it was necessary to use larger quantities of the drier soils before growth was obtained in the broth.

From the results of this experiment it is evident that the organism may survive in the soil under very dry conditions.

It remained viable in soils dried eight months over concentrated sulphuric acid. At the end of this period the moisture contents of the two soils were only 0.45 and 0.25 per cent. These results contradict the popular assumption that the blackleg pathogene is exceptionally sensitive to desiccation. They are, however, in accord with those of Giltner and Langworthy

(5) who found nonspore-forming bacteria surviving in soil moisture of less than one per cent. It seems extremely unlikely that any agricultural soil would in nature become so dry that the blackleg pathogene could not survive.

In comparison with the soil tests, a parallel series of tests were made with the silk-thread method described by Peltier (16) in his study of *Pseudomonas citri*. It was found that the bacteria were killed in less than 24 hours in all degrees of relative humidity under 100 per cent. Goss (6), in 1923, reported briefly that "Investigations on the viability of *B. phytophthorus* under various conditions of temperature and moisture have shown the organism to be very susceptible to desiccation. At 100 per cent humidity the organism remains viable for at least 8 days at all temperatures from 5° to 30° C. At 90 per cent humidity with a temperature of 25° C. it remained viable for only 3 hours. At 80 per cent humidity with the same temperatures the organism lived for only one hour." By personal correspondence it was learned that Goss used the silk-thread method to obtain these results. The writer's results obtained by this method agree with those reported by Goss, but it is evident that the results obtained with its use are not a reliable indicator of the resistance of the bacteria to desiccation in the soil.

It is difficult to say just what the factors are which enable the organism to survive in such extremely dry soil. Giltner and Langworthy think that the traces of hygroscopic moisture ever present about the soil particles are partly responsible, but they think there must be other factors. The water held by the soil colloids must certainly be of some importance. The very slow rate of drying in soils, which allows the bacteria time for becoming adapted to the dry conditions, is also a factor not to be overlooked.

The facts here presented force one to the conclusion that the blackleg pathogene can and does survive in the soil and is possibly very wide-spread in its occurrence, although no extensive survey has been made. When one considers this in connection with what has been demonstrated concerning the methods of infection in potato blackleg, it is realized that the incidence of the pathogene may not be so important in the development of the disease as are the proper conditions for inoculation and infection.

Kotila and Coons (9) have demonstrated by soil- and water-culture inoculations that "the usual method of blackleg infection is not through either injured or uninjured roots." The writer has repeated and verified these experiments. He has also examined hundreds of plants affected with blackleg in all stages of development, with the view of determining the avenue of infection; and, in more than ninety-nine per cent of the cases observed, the infection originated in the seed piece. As has been shown in a

previous paper (11), infection may be systemic in some cases, the seed tubers having been produced on diseased plants and infected through the decaying stolons. In other cases it can be proven that this type of infection is not involved. In some of these cases the seed-corn maggot has been shown by Leach (10) and Bonde (3) to play an important part as a common and effective agent of inoculation. It has been shown also that the insect can and frequently does disseminate the pathogene with its eggs. It appears from the facts presented here that this insect might also pick up the organism from the soil and successfully inoculate the seed pieces. On the other hand, although the pathogenic bacteria may be in the soil in great abundance, they usually are unable to infect the seed pieces without the aid of some such agent of inoculation, which enables them to penetrate the layer of wound cork formed by the seed piece.

If the conclusion that the blackleg pathogene survives in the soil is correct we would expect seed pieces planted under conditions inhibitory to cork formation to be more subject to the disease than those planted under more favorable conditions. There is abundant evidence to show that this is true. It is a matter of common observation that blackleg is most destructive in heavy, moist soils.

Morse (12) states that "more blackleg is observed in wet than in dry seasons" and that "all other things being equal, the disease is more likely to occur in wet than in dry soil, and is more prevalent when the early part of the growing season is characterized by abundant rainfall." He also describes one example in which the disease was first noted "near the center of the affected area from which it gradually spread outward. The season had been excessively wet, and this area coincided with a low, undrained pocket or depression in the field, where water would stand for a few hours after each heavy rainfall."

Murphy (13) says "more diseased plants are found in low-lying parts of a field than in higher." One example is given in which "It was found in extensive counts that there were three blackleg plants in the low-lying area to one in the higher."

The writer has made numerous observations of similar nature, and any one who has studied the disease to any extent will agree that outbreaks of the disease very frequently occur in water-logged soils. Where attempts have been made to explain this relationship, emphasis has been put on the growth requirements of the pathogene and very little attention has been paid to the influence of such conditions on the seed tubers. As pointed out above, it has been assumed generally that the pathogene is very susceptible to desiccation and that such moist soils were especially conducive to its growth. Without doubt such conditions are favorable to the growth of the pathogene

which is a facultative anaerobe, but, at the same time, one should not overlook the influence of water-logged soils on the formation of wound cork by the seed pieces. That abundant oxygen is necessary for the development of cork in the potato tuber has been shown by Kny (8), Appel (2), Olufsen (14), and others. It is obvious that excessive moisture in the soil inhibits cork formation, but apparently there are no records of a careful study of the phenomenon.

In order to obtain a more accurate measure of this influence, freshly cut Early Ohio tubers were planted in a series of jars or cylinders of quartz sand, each of which was held at a different moisture content. In the jars with the higher moisture contents, water was supplied from the bottom, and the differences in water content were obtained by varying the water level. In the drier jars, the water was supplied through Livingston atmometers buried in the center of the jar, sealed, and connected to a bottle of water by a rubber tube. The differences in moisture content were obtained by varying the height of the jar above the bottle of water. On the 1st, 2nd, 4th, 6th, and 8th days after planting, a tuber was removed from each jar and the cut surface was examined for the presence of wound cork. Sections were cut on a sliding microtome, stained in Sudan III, and mounted in glycerine for microscopic examination. In this way a very good idea of the effect of excessive soil moisture on the cork formation was obtained.

It is realized that the conditions of the experiment were not comparable in all respects to those in nature, for the influence of a given moisture content would vary with the type of soil. The results obtained show only the relative effect of different degrees of moisture and not the absolute effect of a given moisture content.

The experiment was made during the month of March when the tubers were no longer dormant. The results, as indicated by the amount of cork formed by the eighth day, are given in table 1.

The progress of healing as observed in the experiment agreed closely with the process described by previous investigators. During the first 48 hours the walls of the outer layer of cells became infiltrated with a dark brown substance which apparently makes them impervious to water. These cells do not readily stain with suberin stains. This process, sometimes termed "blocking," is considered a form of suberization and is inhibited by exclusion of oxygen. Beginning about the third day, under favorable conditions, the starch disappears from and cross walls are formed in the second or third layer of cells beneath the wounded surface. These walls all lie parallel to the wounded surface. It usually requires from 8 to 10 days for the formation and suberization of these new cells which make up the new periderm. It will be noted that in this experiment an excessive amount of

TABLE 1.—*The influence of the amount of moisture in the soil on the amount of wound cork formed by cut potato tubers in eight days*

Pot no.	Moisture content in per cent	Outer cells	New cross walls	
			No.	No. suberized
1	Less than 1	Suberized	2–4	2
2	2.39	do	5–8	3
3	5.91	do	5–8	3
4	7.88	do	5–8	3
5	9.38	do	4–6	2
6	12.03	Lightly suberized	1–4	None
7	12.59	do	1–4	do
8	14.75	Not suberized	None	Outer cells autolyzed

moisture resulted in a decided retardation or complete inhibition of these processes. In the more moist cylinders, not only was there no suberization or cell division, but the cells became soft and mushy, evidently because of asphyxiation and resulting autolysis.

Since the blackleg pathogene is a faculative anaerobe and may survive in the soil, it would seem that under such conditions infections could easily arise from the soil. It is very probable that such infection is largely responsible for the greater abundance of blackleg in excessively wet soils and in unusually rainy seasons. Of course, it is realized that the problem is not so simple as a consideration of these factors alone would imply. Certainly there are other factors which, acting under different conditions, would be of primary importance in determining the development of the disease. These are, however, not considered in the scope of this paper.

The marked influence of oxygen on wound-cork formation and on infection by the blackleg pathogene was further demonstrated by the following experiment. Several potato tubers were inoculated with a pure culture of the blackleg pathogene by the "hole and plug" method. The inoculated tubers were divided into three lots and placed in three separate moist chambers. The first container was a 3-gallon earthenware crock with a fairly loose-fitting top. The moisture was supplied by a layer of wet cotton on the bottom of the crock. The second was a large glass desiccator with a close-fitting top. The lower section was filled with water, and the inoculated tubers were suspended on a wire platform above the water. The third container was similar to the second but differed in that a stream of fresh air was drawn through the desiccator by means of two glass tubes sealed in the top of the vessel. The air-inlet tube extended to a point just below the tubers so that the air was drawn about the tubers and then removed from

the top. The air current was supplied by a suction pump and the air was bubbled through two flasks of water before it was drawn into the moist chamber. Saturation of the air in the chamber was insured by keeping the water in these flasks a few degrees warmer than the temperature in the chamber. In this way the warm, moist air was slightly cooled on entering the chamber and precipitated moisture was visible on the sides of the vessel throughout the duration of the experiment. The moist chambers were placed side by side on a table at room temperature.

In the first container the loose-fitting top permitted sufficient interchange of air to cause some fluctuation and variation in relative humidity, although it probably closely approached saturation at all times. The oxy-

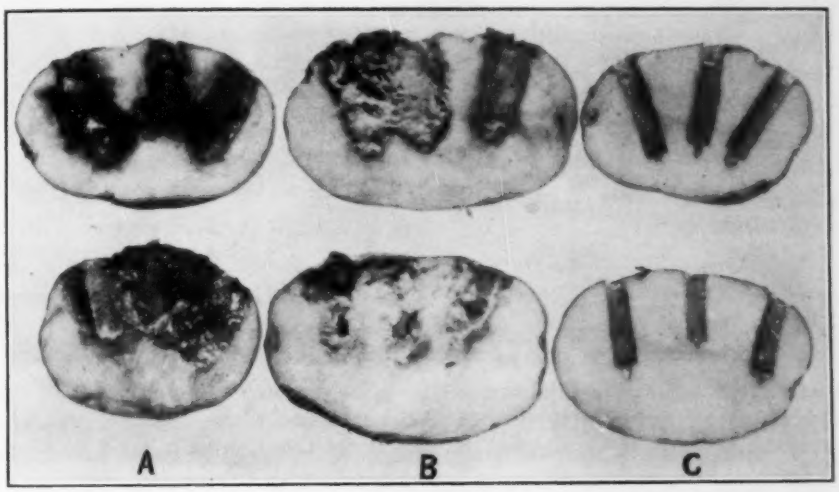

FIG. 1. The influence of oxygen supply on infection and decay of potato tubers by the blackleg pathogene. A. Tubers incubated in moist chamber with loose-fitting top. B. Tubers incubated in moist chamber with close-fitting top. C. Tubers incubated in a moist chamber in saturated atmosphere but with a constant supply of fresh air.

gen supply in the crock was soon consumed by the respiring tubers and was not entirely replaced by diffusion through the leak in the top.

In the second container the air was saturated with moisture at all times, but the oxygen consumed in respiration could not be replaced.

In the third container a saturated relative humidity combined with abundant oxygen supply prevailed.

After four days of incubation two tubers were removed from each container and cut in half to determine the amount of decay. These tubers are shown in figure 1. It will be seen that the tubers in the first two containers were badly decayed, while those in the container receiving a constant sup-

ply of fresh air had not decayed at all. The decayed tissue of the tubers incubated in the closed desiccator was almost pure white, showing only traces of browning along the margins. The influence of the limited supply of oxygen available in the crock was evident in the darker color of the decayed tissue of the tubers incubated in the crock.

Microtome sections were cut through the inoculated portions of the tubers shown in figure 1 and were examined for wound cork. Photographs of these sections are shown in figure 2. It was found that an abundance of

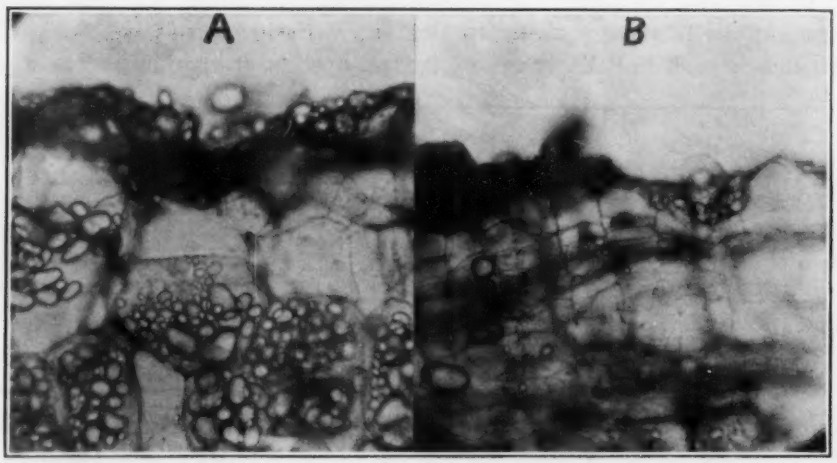

Fig. 2. The influence of oxygen supply on wound-cork formation by tuber inoculated with the blackleg pathogene and incubated in moist chambers. A. A photomicrograph of a section of the infection court of the tubers shown in figure 1, A. B. The same for figure 1, C.

wound cork had been formed by the tubers incubated in a constant supply of oxygen but that none had formed in the other tubers.

This experiment was repeated several times and it was found to be almost impossible to rot tubers with the blackleg pathogene when they were given a constant supply of fresh air, even when incubated at the optimum temperature and in a saturated atmosphere.

The influence of oxygen on infection and development of blackleg decay, as brought out in this experiment, should be of some practical importance in the storage of potatoes. It is limited, however, in its application by the fact that the constant replacement of oxygen stimulates respiration and the resulting sprouting of the tubers.

An appreciation of this relationship is also of significance in experimental work. It has been observed, for example, that when a single tuber is inoculated and placed alone in a large moist chamber it will frequently

escape infection. On the other hand, if the moist chamber is filled with tubers so that the oxygen supply is soon exhausted by respiration, practically 100 per cent infection is obtained. A failure to recognize this influence of the oxygen supply may have been responsible for many of the variable results reported in literature dealing with bacterial decay of potato tubers.

SUMMARY

1. The pathogene causing potato blackleg can and commonly does survive the winter in the soil in Minnesota.

2. The conclusion of earlier workers that this organism could not survive the winter in the soil is not justified. Their conclusion was based on negative evidence which loses its force in the light of recent investigations on the methods of infection.

3. The pathogene is resistant to the very low temperatures of a Minnesota winter.

4. Experiments in which the organism was dried slowly in soils proved it to be extremely resistant to desiccation. The conditions of these experiments are considered to be more comparable to those occurring in nature than those in which the bacteria are dried on cover glasses or silk threads.

5. Excessive soil moisture greatly inhibits the formation of cork. This inhibition is probably due to the exclusion of oxygen. The blackleg pathogene, being a facultative anaerobe, grows readily under such conditions. It is concluded that under such conditions blackleg may arise by infection from the soil. It is also concluded that the inhibition of cork formation by the exclusion of oxygen is largely responsible for the more abundant development of blackleg in excessively wet soils or in very rainy seasons.

6. All the evidence obtained by a study of potato blackleg over a series of years leads to the conclusion that the presence or absence of the proper conditions for infection, or of some agent of inoculation, may be of greater importance as a limiting factor in the development of the disease than the incidence of the pathogene.

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PHYSIOLOGIC FORMS OF BARLEY MILDEW, *ERYSIPHE GRAMINIS HORDEI* MARCHAL

E. B. MAINS AND S. M. DIETZ[1]

Physiologic specialization of rusts of grasses has long been known. However, it was not until 1902 that Marchal (2) first pointed out the existence of physiologic specialization in the powdery mildew (*Erysiphe graminis* DC.) of grasses.

He distinguished seven races of *Erysiphe graminis* based on their host specialization: *Tritici* on species of Triticum, *Hordei* on species of Hordeum, *Secalis* on species of Secale, *Avenae* on species of Avena, *Poae* on species of Poa, *Agropyri* on species of Agropyron, and *Bromi* on species of Bromus. He found that conidia of the mildew from *Hordeum vulgare* would infect *Hordeum vulgare, H. hexastichon, H. trifurcatum, H. distichon, H. Zeocriton, H. nudum, H. jubatum, H. murinum* but not *Agropyron repens, A. caninum, A. giganteum; Agrostis alba, Alopecurus pratensis, Andropogon Ischaemon, Anthoxanthum odoratum, Aira caryophyllea, Avena sativa, A. orientalis, A. fatua; Arrhenatherum elatius Brachypodium sylvaticum, Briza media, Bromus sterilis, B. patulus, B. mollis, B. racemosus, B. secalinus, B. arduennensis, B. squarrosus, B. macrostachys, Cynosurus cristatus, Dactylis glomerata, Deschampsia flexuosa, Elymus arenarius, Festuca rubra, F. elatior, F. gigantea; Holcus lanatus, Koehleria cristata, Lolium perenne, Melica ciliata, Milium effusum, Phleum pratense, P. Boehmeri; Poa annua, P. nemoralis, P. serotina, P. pratensis, P. mulalensis, P. trivalis; Secale cereale, Setaria viridis, Trisetum flavescens, Triticum vulgare, T. Spelta, T. polonicum.* Similar results (3) were obtained when ascospores were used.

Both Reed and Salmon have studied the race *Hordei.* Salmon (5) was able to infect *H. decepiens, H. distichon, H. hexastichon, H. intermedium, H. vulgare,* and *H. Zeocriton.* A slight infection was obtained on *H. bulbosum* and *H. maritimum.* He was not able to infect *H. jubatum, H. murinum, H. secalinum, H. silvaticum, Avena sativa, Triticum vulgare,* nor *Secale cereale.* Reed (4) infected *H. distichon* L., *H. nudum* L., *H. steudelii* x *trifurcatum, H. tetrastichon* L., *H. trifurcatum* Jacq., *H. vulgare* L., *H. Zeocriton* L., and young seedlings of *H. nodosum* L. Negative results were obtained with *H. bulbosum* L., *H. maritimum* With., *H. jubatum* L., *Triticum vulgare* Vill., *T. dicoccum* Schrank, *Triticum durum*

[1] Published with the approval of the Directors as a joint contribution from the Department of Botany of the Purdue University Agricultural Experiment Station, LaFayette, Ind., and the Section of Botany and Plant Pathology, Iowa Agricultural Experiment Station, Ames, Iowa.

Desf., *Avena sativa* L., and *Secale cereale* L. From the studies of Marchal, Salmon, and Reed, therefore, it would appear that the race *Hordei* is rather closely restricted to a few closely related species of Hordeum.

Salmon and Reed made their studies with the powdery mildew from various species of Hordeum. They found little evidence of differences in pathogenicity of the mildew from such different sources. Their results, therefore, would indicate that the *Hordei* race was rather uniform in its pathogenicity. They, however, apparently did not study *Erysiphe graminis hordei* from a number of different localities. Apparently, their studies were for the most part confined to relatively few varieties of barley.

Salmon, however, devoted some attention to the question of whether any of the species or varieties of barley showed differences in susceptibility to mildew. In studies conducted in the greenhouse where plants were inoculated under bell-jars only the botanical varieties *Hordeum vulgare* var. *leiorrhynchum* and *H. vulgare* var. *himalayense* showed any signs of resistance. He found from a study under field conditions in 1903 when powdery mildew was abundant at Cambridge, England, that *Hordeum vulgare* var. *himalayense, Walpersii, crispum; H. Zeocriton* var. *melanzeocriton; H. distichon* var. *pictum, eingens, medicum, persicum, laxum, ianthinium, ramulosum, nigrosubinerme, Rimpaui, H. decepiens* var. *triceros, nudideficiens; H. hexastichon* var. *hexasticho-ramosum; H. spontaneum* showed no mildew. Some varieties showed only a trace, while others developed a fair amount and still others were excessively mildewed.

MATERIALS AND METHODS

The studies described in this paper are an outgrowth of extensive studies of varieties of barley for reaction to leaf rust, *Puccinia anomala,* which are cooperative investigations between the Office of Cereal Crops and Diseases of the United States Department of Agriculture and the Botanical Department of the Purdue University Agricultural Experiment Station. During the winter of 1923–1924, 655 selections and varieties received from the Office of Cereal Crops and Diseases were studied in the greenhouse of the Agricultural Experiment Station of Purdue University for reaction to leaf rust. Considerable mildew developed on these varieties during the study for rust reaction and it was noted that apparently marked differences occurred in the reaction of varieties to mildew. Therefore it was decided to inoculate all the varieties with mildew after notes concerning rust had been obtained. As a result complete notes were obtained concerning both rust and mildew and marked differences in the reactions were found to exist.

In 1925, 1926, 1927, and 1928 these studies were continued on a selected series of varieties at Purdue University. In 1926 a selected set of

varieties was furnished the Section of Botany and Plant Pathology of the Iowa Agricultural Experiment Station for similar studies of powdery mildew where studies were made in 1926, 1927, and 1928.[2]

It is not proposed in this paper to attempt to give all of the data accumulated during the five years. One of the results of this study has been the discovery of the existence of physiologic forms, which may be distinguished by the use of a few of the varieties studied. These, together with a few others, outstanding for their general resistance or susceptibility to the various cultures of mildew, have been selected for discussion.

These varieties were grown in four-inch pots in the greenhouse during the winter months and were inoculated in the second- to fourth-leaf stage of development. The cultures of mildew were carried on some susceptible variety, such as Oderbrucker, C. I. 940.

The varieties were inoculated by shaking potted plants of heavily-mildewed-susceptible varieties over pots of seedlings of the varieties to be tested, thus dusting such seedlings with conidia. The seedlings were then atomized with a fine spray of water and covered with wet muslin-cloth or placed in a moist chamber for 24 hours. Notes were taken when the development of the mildew had reached its maximum, usually in 7 to 14 days after inoculation.

Five classes of reaction were distinguished, as in the case of the powdery mildew of rye (1). These classes are as follows:

Highly resistant, denoted as 0. Macroscopically, no mycelium is evident. Chlorotic or necrotic spots may be developed by some varieties. Microscopically, a slight amount of mycelium may develop and infection may be evident macroscopically by faint flecks. (Fig. 1, A–C.)

Very resistant, denoted as 1. Slight to moderate development of mycelium evident macroscopically but with little or no sporulation. (Fig. 1, D and E.) Chlorotic or necrotic spots developed by some varieties.

Moderately resistant, denoted by 2. A moderate to abundant development of mycelium occurs, accompanied by a slight production of conidia, figure 1, F and G. Chlorotic or necrotic areas are formed by some varieties.

Moderately susceptible, denoted by 3. A moderate to abundant development of mycelium occurs, accompanied by moderate sporulation. Figure 1, H.

Very susceptible, denoted by 4. Abundant mycelium is developed, accompanied by abundant sporulation. Figure 1, I and J.

[2] The writers are indebted to Dr. H. V. Harlan, who brought this extensive collection of varieties together from various parts of the world. We also wish to express our thanks to Dr. M. N. Pope, who kindly furnished us with the seed, and to Miss Mary L. Martini, who has furnished information concerning the classification of the varieties, and, further, to Mr. Leroy Compton, Mr. L. D. Leach, and Mr. H. C. Murphy, who assisted in the inoculations at Purdue University and Iowa State College.

RESULTS

During the winter of 1923–1924, the culture of mildew occurring in the greenhouse of the Department of Botany, Purdue Agricultural Experiment Station at LaFayette, Indiana, was studied on a large series of varieties. During the winter of 1924–1925, mildew in the same greenhouses was again studied on a selected set of varieties. The results obtained agreed very closely with those of the previous year. Twice during the winter of 1925–1926 and again in 1926–1927 the selected varieties were tested to mildew carried over from the previous year and results very similar to those of the previous years were obtained. These are summarized in table 1 under physiologic form 1. The varieties Arlington 702, Goldfoil 928, Hanna 906, C. I. 2444, and C. I. 1080 were outstanding for their marked resistance, being consistently of type 0. The varieties Kwan 1016 and Duplex 2433 showed a slight development of mycelium but no sporulation and are classed as type 1–. Consul 1061, Oswong 697, and C. I. 2416 showed still more development of mildew, being, however, very resistant, type 1. Some of the varieties showed more variation in reaction than others. Such varieties as Chilean D, Peruvian 1131, Turkestan 711, Bolivia 1257, Coast 276, Common Chile 663, Chilean C 1432, Abyssinian 1243, Luth 972, Peruvian 935, Lynch 919, and C. I. 1021 varied in reaction from type 0 to 1. Abyssinian 362, Hanna 966, Purple Nepal 1373, Black Hullless 1032, Nepal 595, and Heil's Hanna 682 varied from 1 to 2. Palestine 939, Blackhull 878, Arequipa 1256, Peru 653, Lehor 866, and Black Hullless 666 showed more variation, sometimes giving a reaction of type 0 and, at other times, type 1 or 2. Juliaca 1114 varied from type 2–3 and Nepal 475 from type 1–3. Oderbrucker 940 and Hooded Spring 716, while susceptible, showed some variation from type 3–4. Odessa 182 and Bohemia 204 represent a large number of highly-susceptible varieties, showing a consistent reaction of type 4.

This culture of mildew was lost during the summer of 1927 and no barley mildew occurred in the greenhouses at LaFayette, Indiana, during the winter of 1927–1928. In the autumn of 1928 powdery mildew was found on some plants of volunteer barley at LaFayette, Indiana, and was again studied upon the series of varieties used previous years. To this culture, several of the varieties showed considerably more susceptibility than to the cultures of previous years. The results of three tests during the winter of 1928–1929 are given in table 1 under physiologic form 2. Most of the varieties gave a reaction to form 2 very similar to that to form 1. However, Oswong 697, Purple Nepal 1373, Black Hullless 1032, Nepal 595, Nepal 475, and Heil's Hanna 682 showed considerably more susceptibility than to physiologic form 1, as can be seen by consulting table 1.

TABLE 1.—*Reaction of forty varieties of barley to five physiologic forms of powdery mildew, Erysiphe graminis hordei*

Variety[a]	C. I.[b] No.	Type of reaction to				
		P. f. 1	P. f. 2	P. f. 3	P. f. 4	P. f. 5
Hordeum vulgare pallidum						
Duplex	2433	1–	0	0	0	0
Kwan	1016	1–	0	0–1	0–1	1–2
Chilean D	1433	0–1	1	1	2	0–1
	1021	0–1	0	0	2	2
Coast	276	0–1	1–2	1	2	1
Chilean C	1432	0–1	1+	1+	2	1
Abyssinian	1243	0–1	2	1+	2	1
Common Chile	663	0–1	1+	2+	1–2	0–1
Peruvian	1131	0–1	0–1	2–3	1	3
Turkestan	711	0–1	0–1	2–3	1	2–3+
Bolivia	1257	0–1	1	1+	2–3	2–4
Luth	972	0–1	0–1	2–3	1	2–3
Consul	1061	1	1	0	3	1–2
Peruvian	935	0–1	1	3–4	1	4
Lynch	919	0–1	0–1	3–4	1	3–4
Arequipa	1256	0–2	1	3–4	2	4
Peru	653	0–2	1–2	1+	3	3–4
Juliaca	1114	2–3	2+	1+	4	4
Oderbrucker	940	3–4	4	4	4	4
Odessa	182	4	4	4	4	4
Hordeum vulgare nigrum						
Oswong	697	1	3	3–4	2	3
Hordeum vulgare horsfordianum						
Hooded Spring	716	3–4	3–4	4	4	4
Hordeum vulgare coeleste						
Black Hullless	666	0–2	1–2	3+	4	3
Hordeum vulgare trifurcatum						
Lehor	866	0–2	2	2–3	4	3–4
Purple Nepal	1373	1–2	3+	4	4	3–4
Black Hullless	1032	1–2	3+	3+	4	4
Nepal	595	1–2	4	4	4	4
Nepal	475	1–3	4	4	4	4
Hordeum intermedium haxtoni						
Arlington	702	0	0	0	1	0
	1080	0	1+	0	3	1–2
Hordeum intermedium mortoni	2444	0	0	0–1	0–1	1
Abyssinian intermediate	2416	1	1+	1+	2	3–4
Hordeum distichon palmella						
Palestine	939	0–2	1	1	1	1
Goldfoil	928	0	0	0	0	4
Hanna	906	0	0	0	0	4
Hanna	966	1–2	1	3	0	3–4
Heil's Hanna 3	682	1–2	4	4	3–4	4
Bohemia	204	4	4	4	4	4
Hordeum deficiens steudeli						
Abyssinian	362	1–2	1+	1	2	1
Blackhull	878	0–2	1	3–4	1	4

[a] Varieties classified according to H. V. Harlan. The Identification of Varieties of Barley, U. S. Dept. Agr. Bul. 622. 1918.

[b] Accession number of the Office of Cereal Crops and Diseases, United States Department of Agriculture.

In the autumn of 1928 a collection of barley mildew was received from Dr. J. G. Dickson from Madison, Wisconsin. This was carried in a greenhouse compartment separate from the preceding and sown upon a similar set of varieties. Three different tests were made and the results showed this to be a physiologic form distinct from forms 1 and 2. The results of these studies are given under physiologic form 3 of table 1. As can be noted, this form is sharply separated from physiologic forms 1 and 2 by the reaction of Peruvian 935 and Lynch 919 (Fig. 2, A3 and B3), which showed a reaction of type 3 or 4 as contrasted with the types 0–1 shown to physiologic forms 1 and 2. The varieties Hanna 966 (Fig. 2, A4 and B4), Blackhull 878, Arequipa 1256 (Fig. 1, I), and Black Hullless 666 showed considerably more susceptibility to physiologic form 3 than to either of the other forms, and the varieties Oswong 697, Purple Nepal 1373, Black Hullless 1032, Nepal 595, Nepal 475, and Heil's Hanna 682 (Fig. 1, J) were considerably more susceptible than to physiologic form 1, but approached physiologic form 2 in their reactions.

In 1926 a collection of powdery mildew was made at Moscow, Idaho, and studied in the greenhouses of the Section of Botany and Plant Pathology of the Iowa Agricultural Experiment Station on the varieties used in the studies at Purdue University. This culture proved to be distinct from the three forms previously studied. In 1927 a collection was made in California, which proved to be very similar to the collection from Idaho. The results obtained from these studies are given in table 1 under physiologic form 4. The varieties Juliaca 1114, Lehor 866, Peru 653, Consul 1061, and C. I. 1080 were more susceptible to this form than to physiologic forms 1, 2 and 3. The reaction of Peruvian 935, Lynch 919, Blackhull 878, and Arequipa 1257 to form 4 resembled their reaction to physiologic forms 1 and 2, and they were more resistant to form 4 than to form 3. Hanna 966 showed considerably more resistance to form 4 than to forms 1, 2, and 3. Heil's Hanna 682, on the other hand, was more or less susceptible, types 3–4, to form 4, thus showing a reaction similar to its reaction to forms 2 and 3 but differing from its reaction to form 1 to which it was resistant, types 1–2.

In the summer of 1928 a culture of barley mildew was obtained at Ames, Iowa, which gave markedly different reactions in studies made during the winter of 1928–1929 at Iowa State College. The results of this study are given in table 1 under the heading physiologic form 5. This physiologic form is easily separated from the other four forms by the reactions of Goldfoil 928 and Hanna 906 (Fig. 3), which were very susceptible, type 4, to physiologic form 5 and highly resistant, type 0, to all the other forms. The unnamed variety C. I. 2416 was also much more susceptible to form 5 than to the other forms. Peruvian 935, Lynch 919, Blackhull 878, and Arequipa

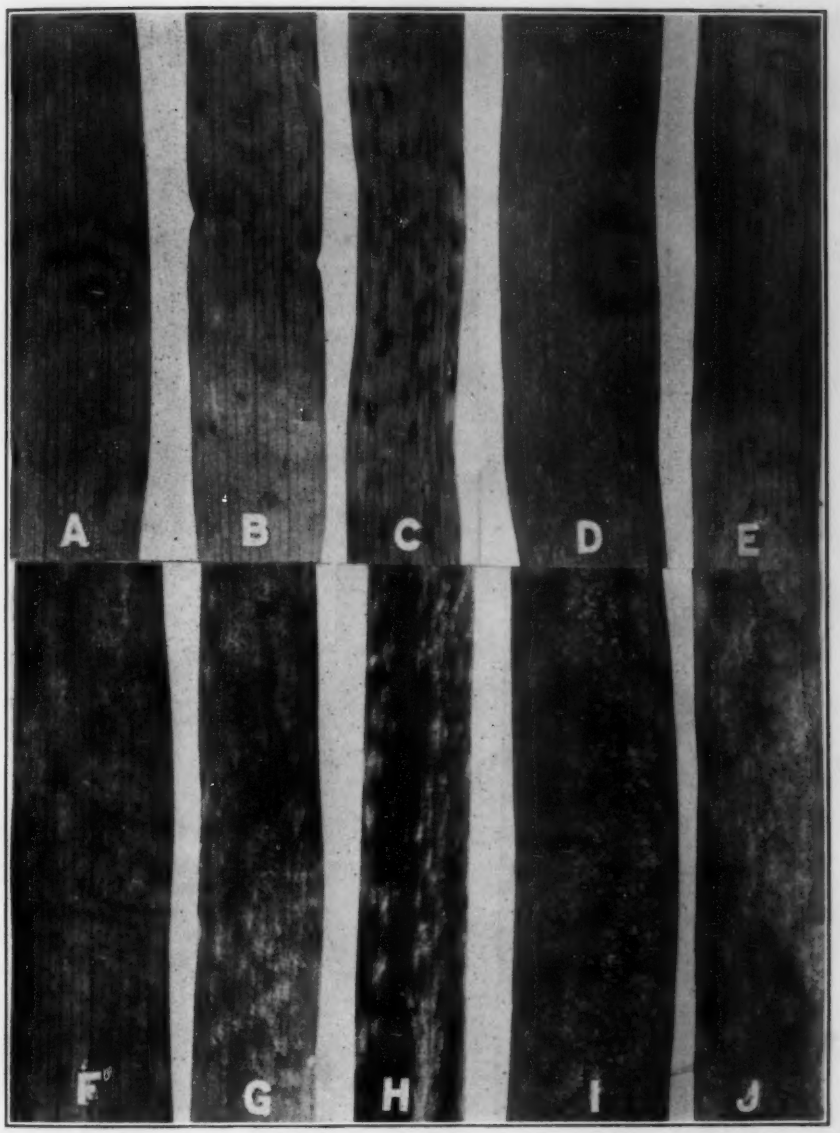

FIG. 1. Types of reaction of barley varieties to *Erysiphe graminis hordei* p. f. 3. A and B. Type 0, highly resistant, slight flecking. C. Type 0, highly resistant with definite necrotic spots. D and E. Type 1, very resistant, slight development of mycelium, and very slight sporulation. F and G. Type 2, moderate development of mycelium and slight sporulation. H. Type 3, moderately susceptible, moderate development of mycelium, and moderate sporulation. I and J. Very susceptible, abundant development of mycelium and sporulation. A. Arlington C. I. 702; B. Consul C. I. 1061; C. C. I. 1080; D. Abyssinian C. I. 362; E. Palestine C. I. 939; F. Luth C. I. 972; G. Peruvian C. I. 1131; H. Hanna C. I. 966; I. Arequipa C. I. 1256; J. Heil's Hanna No. 3, C. I. 682.

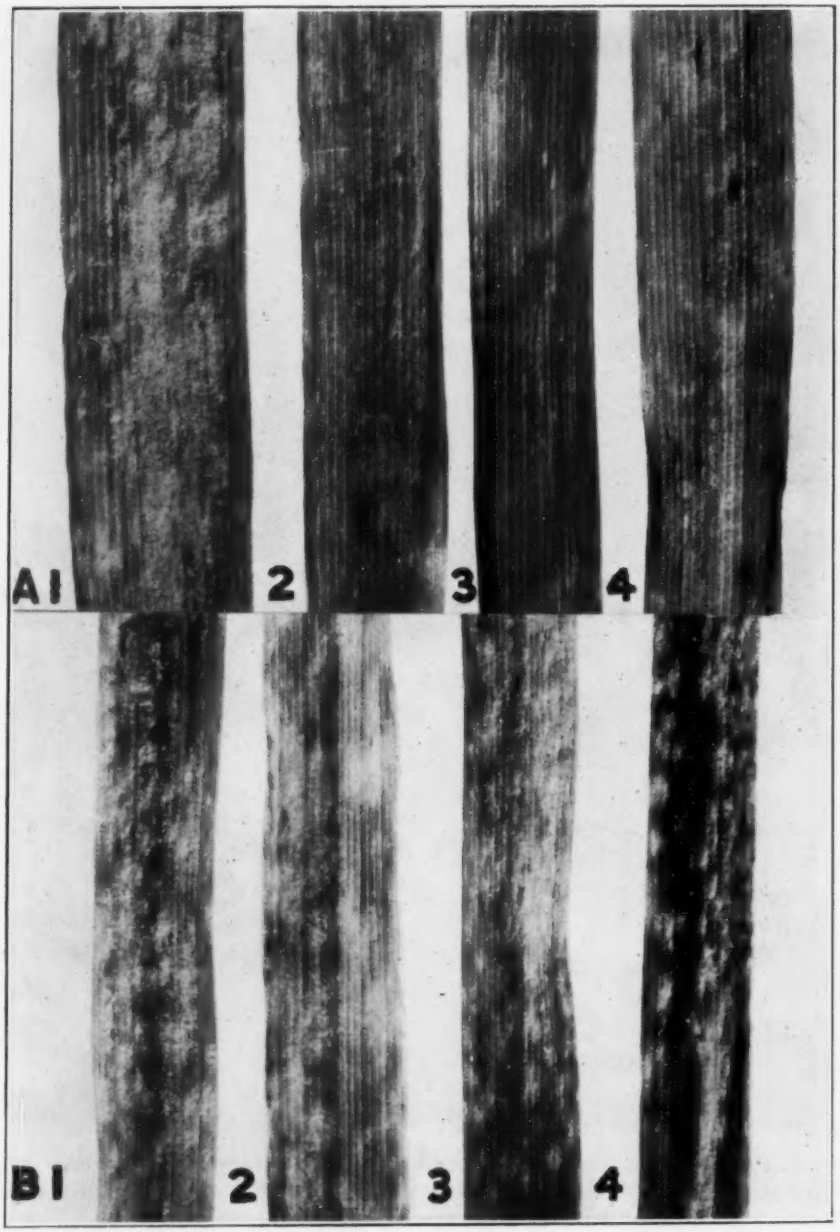

FIG. 2. Reaction of four varieties of barley to two physiologic forms of powdery mildew. A. Four varieties inoculated with physiologic form 2. B. Same four varieties inoculated with physiologic form 3. (1) Oderbrucker C. I. 940, susceptible to both forms. (2) Turkestan C. I. 711, highly resistant to p. f. 2 and moderately resistant to p. f. 3. (3) Lynch C. I. 919, highly resistant to p. f. 2 and susceptible to p. f. 3. (4) Hanna C. I. 966, very resistant to p. f. 2 and moderately susceptible to p. f. 3.

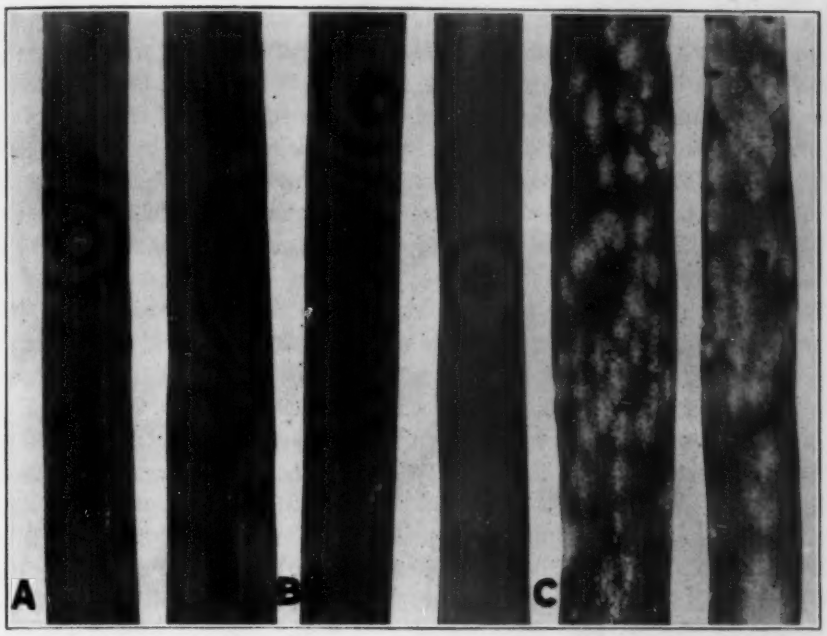

FIG. 3. Reaction of Hanna C. I. 906 to three physiologic forms of powdery mildew. A. Two leaves of Hanna inoculated with p. f. 2, highly resistant. B. Two leaves inoculated with p. f. 3, highly resistant. C. Two leaves inoculated with p. f. 5, very susceptible.

1256 were susceptible to form 5, thus giving a reaction similar to that to form 3 and differing from that to forms 1, 2, and 4 to which these varieties were resistant. Consul 1061 and C. I. 1080 were more resistant than to form 4 and somewhat less resistant than to forms 1, 2, and 3. Peru 653, Juliaca 1114, and Lehor 866, which were more or less resistant to forms 1, 2, and 3, were susceptible to both 4 and 5. Black Hullless 666 was more or less susceptible to forms 3, 4, and 5 and resistant to forms 1 and 2. Purple Nepal 1373, Black Hullless 1032, Nepal 595, Nepal 475, and Heil's Hanna 682 were resistant only to form 1 and susceptible to the other forms.

The five physiologic forms can be most easily separated by using four barley varieties as follows:

Nepal C. I. 595 resistant (1–2)	p. f. 1
Nepal C. I. 595 susceptible (4)	
Peruvian C. I. 935 susceptible (3–4)	
Goldfoil C. I. 928 susceptible (4)	p. f. 5
Goldfoil C. I. 928 resistant (0)	p. f. 3
Peruvian C. I. 935 resistant (0–1)	

Black Hullless C. I. 666 resistant (1–2) p. f. 2
Black Hullless C. I. 666 susceptible (4) p. f. 4

As shown in table 1, the varieties Arlington 702, Duplex 2433, and C. I. 2444 are outstanding for their marked resistance to all five of the physiologic forms. These varieties are closely followed in resistance by Kwan 1016, Chilean D 433, C. I. 1021, Peruvian 1131, Turkestan 711, Bolivia 1257, Palestine 939, Abyssinian 362, Coast 276, Common Chile 663, Chilean C 1432, Abyssinian 1243, and Luth 972, varieties more or less resistant to all five of the physiologic forms.

The varieties Goldfoil 928 and Hanna 906 are very resistant to four physiologic forms and susceptible to one. Consul 1061 and the unnamed varieties C. I. 1080 and C. I. 2416 are more or less resistant to four physiologic forms and susceptible to one. Peruvian 935, Lynch 919, Blackhull 878, Arequipa 1256, Hanna 966, Peru 653, Juliaca 1114, and Lehor 866 are more or less resistant to three physiologic forms and more or less susceptible to two. Black Hullless 666 and Oswong 697 are more or less resistant to two physiologic forms and susceptible to 3. Purple Nepal 1373, Black Hullless 1032, Nepal 595, Nepal 475, and Heil's Hanna 682 are resistant to only one physiologic form and more or less susceptible to the other four. Oderbrucker 940, Odessa 182, Bohemia 204, and Hooded Spring 716 are included as examples of a large number of varieties that are susceptible to all of the physiologic forms.

The varieties resistant to all five of the physiologic forms are not restricted to any one species or group. They are distributed in all the major species, *Hordeum vulgare, Hordeum intermedium, Hordeum distichon,* and *Hordeum deficiens,* table 1. A similar situation also occurs even in agronomic varieties, as is shown by the reactions of the various selections of Hanna to physiologic form 3, Hanna 906 registering a type 0 reaction, Hanna 966 giving a reaction of type 3, and Heil's Hanna 682 giving a reaction of type 4.

DISCUSSION

These studies show that the various species of barley, *Hordeum vulgare, H. intermedium, H. distichon,* and *H. deficiens* are not uniformly favorable hosts for *Erysiphe graminis hordei.* While a large number of susceptible varieties occur in all of these species, a number of resistant varieties also are found in each. These resistant varieties also differ considerably. Some show no macroscopic signs of infection; some allow a slight development of mycelium with little or no sporulation; while others develop a slight to moderate sporulation.

Not only is it evident that the species *Erysiphe graminis* can be separated into races by the species of hosts which such races are able to infect,

but that the race *Hordei* can be still further subdivided into physiologic forms. Such physiologic forms can be sharply separated by the reaction of certain selections of barley. On the parasitic relationship, therefore, both the hosts and the parasites can be readily separated into groups by their reactions with each other. A situation, therefore, exists in this group similar to that which occurs in several other groups of parasitic fungi, such as the rusts and smuts.

It is evident that the existence of physiologic forms complicates exceedingly the problem of controlling the powdery mildew of barley with resistant varieties. Before this can be done with any degree of certainty it will be desirable to have the fullest possible knowledge of the extant physiologic forms and of the relative susceptibility of barley varieties not only in the seedling stage but under different environmental conditions and stages of development of the barley plant.

SUMMARY

1. The race of powdery mildew, *Erysiphe graminis hordei,* on barley contains at least five physiologic forms.

2. These physiologic forms can be separated by the reaction of a selected set of barley varieties resistant to some of the physiologic forms and susceptible to the others in the seedling stage in the greenhouse.

3. Barley varieties resistant to these physiologic forms have been found in all of the principal species, *Hordeum vulgare, H. intermedium, H. distichon,* and *H. deficiens.*

4. Of the 40 varieties discussed in this paper, three have been found very resistant to all five of the physiologic forms. Sixteen showed more or less resistance to all five physiologic forms. Five varieties were resistant to four forms and susceptible to one. Eight varieties were resistant to three forms and susceptible to two. Two varieties were resistant to two forms and susceptible to three. Five varieties were resistant to one form and susceptible to four, while four varieties were susceptible to all five physiologic forms.

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CULTURAL CHARACTERISTICS OF PHYSIOLOGIC FORMS OF SPHACELOTHECA SORGHI[1]

C. H. Ficke and C. O. Johnston

INTRODUCTION

The covered kernel smut of sorghum (*Sphacelotheca sorghi* (Link) Clinton) is the most destructive disease of grain sorghums in the southern Great Plains area. Prior to 1923 varieties of milo and feterita seemed to be free from this disease and it was not considered necessary to treat the seed for smut. In 1923 and 1924 specimens of kernel smut were collected in fields of milo in Kansas, New Mexico, and Texas. In 1921 a small percentage of smut was found in feterita at Chillicothe, Texas. Tisdale, Melchers, and Clemmer (11) studied the pathogenicity of three strains of the covered kernel smut, which they designated as the common, milo, and feterita strains. They reported that the common strain attacked many varieties of sorghum, but failed to infect milo and feterita. The milo form infected milo and several other varieties, but failed to infect feterita. The feterita strain infected feterita, but failed to infect milo. Thus they proved that the three strains were distinct and probably were physiologic forms of the fungus causing covered kernel smut.

The studies herein reported were conducted in the laboratory of the Kansas Agricultural Experiment Station during the winter of 1928–29. The results of these experiments show that the three strains mentioned above also can be distinguished by cultural characteristics on various kinds of nutrient media and indicate that covered kernel smut is caused by at least three definite physiologic forms of *S. sorghi*. For convenience these forms have been assigned numbers and reference to them hereafter will be by number. The common covered kernel smut, the milo, and the feterita forms discussed by Tisdale, Melchers, and Clemmer will be referred to as physiologic forms 1, 2, and 3, respectively.

Christensen and Stakman (2) described fifteen physiologic forms of *Ustilago zeae* (Beckm.) Ung. that were distinguishable by such cultural characters as rate of growth, color, topography, surface, zonation, conidial production, and margin. At least seven of these could be recognized by their parasitic behavior on corn. More recently Rodenhiser (8) reported that he was able to distinguish fourteen physiologic forms of *Ustilago tritici* (Pers.) Jens., twelve of *U. nuda* (Jens.) K. and S., seven of *U. hordei*

[1] Contribution No. 298 from the Department of Botany and Plant Pathology, Kansas State Agricultural College, cooperating with the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

(Pers.) K. and S., five of *U. levis* (K. &. S.) Mag., and eighteen of *U. avenae* (Pers.) Jens., in culture by color, topography, surface, consistency, and type of margin. These could be distinguished only on the proper media and under proper environmental conditions.

MATERIALS AND METHODS

Smutted heads from typical plants of Dwarf Yellow milo, feterita, and Pink kafir were selected as sources of smut. Previous experiments in the field had proved that the three strains of smut selected for these studies were pathologically distinct and probably were distinct physiologic forms. The varieties of sorghum on which these forms developed had been inbred for several generations and were known to be pure for varietal characters and reaction to covered kernel smut. As soon as the smutted heads emerged from the boot they were bagged, tagged, and then kept covered until the smut was well developed. At maturity the heads were cut from the stalks without removing the bags and dried. Those infected with each strain of smut were stored separately. There was thus virtually no opportunity for mixture or contamination in the field or in harvesting and storage.

When needed for culturing the heads were removed from the bags one at a time and unruptured smut galls selected on each. There seemed to be no consistent differences in shape, size, or color of the sori produced by the three strains. The enclosing membrane of the smut gall was disinfected and a portion of it carefully removed. A small quantity of the chlamydospores was taken from the interior with a sterile needle. These spores were immediately transferred to test-tubes of liquid carrot agar from which poured plates were made. Some multichlamydospore cultures were also obtained by making direct transfers of spore material from the smut gall to carrot-agar slants. Previous cultural experiments with *U. zeae* and other smuts had shown this medium to be very satisfactory for the culturing of smut fungi. Several multispore cultures were made from each form of the smut in this manner and kept in the laboratory at room temperature.

After a few days' growth there was evidence that the strains under investigation were culturally distinct. Subcultures immediately were made by transferring to fresh plates. At the same time a new series of cultures was made from the smutted heads. These latter proved to have the same general characteristics as the first series of cultures.

It seemed desirable to ascertain whether differences between the forms could be distinguished on media other than carrot agar. Transfers, accordingly, were made to poured plates of potato-dextrose, oatmeal, and carrot agars. As will be pointed out later, distinct differences could be distinguished between all of the forms on the three kinds of agar. All of these operations were repeated several times to make sure that the dif-

ferences were not merely chance variations. With but one exception in form 2, multispore cultures of forms 2 and 3 remained constant for their general cultural characteristics on each of the media. They had somewhat different characters on the various media, however. In short, they seemed to be very stable and to give the same reactions repeatedly. Form 1, on the other hand, seemed relatively unstable and numerous wedge-shape or fan-shape sectors developed in it, especially on carrot agar. These sectors often had characters strikingly different from those of the parent culture.

The origin of the sectors in cultures might be explained on the basis of a mixture of forms, mutations, or heterothallism. Several series of cultures from single sporidia and single chlamydospores, respectively, were made from each of the forms of smut studied. Transfers of each of the forms were made from stock cultures on agar slants to Erlenmeyer flasks of carrot decoction. The cultures thus established were allowed to develop until the medium became cloudy with conidia. Very sparse sowings of conidia then were made in poured plates of carrot agar by the dilution method. Single isolated conidia were located, marked, and transferred to fresh plates as soon as cultures from these were macroscopically visible. Many single conidium cultures were thus established. It has been mentioned that the multichlamydospore cultures were obtained by making direct transfers of spore material from the smut gall to carrot-agar slants. The single chlamydospore cultures were obtained by the dilution plate method. All cultures were kept at room temperature, about 80° F. The single chlamydospore cultures as well as the multispore cultures were grown on carrot agar, potato-dextrose agar, and oatmeal agar. The single conidial cultures, with the exception of those of form 1, were grown on carrot agar only. The cultural characteristics of the three forms on these agars will be discussed later.

One remarkable feature of these experiments was the ease with which *Sphacelotheca sorghi* could be cultured. Several of the smuts have been studied in culture at the Kansas Agricultural Experiment Station, but none seemed to be so easily cultured as *S. sorghi*. It proved to be relatively easy to secure cultures free from contamination and to maintain them without loss of vigor. The simplicity of the methods of culturing described above indicates the facility with which this organism may be handled in culture.

EXPERIMENTAL RESULTS

The fungus taken from wholly or partially smutted heads was grown separately on several artificial media. Constant differences in cultural characteristics between them have been noted during the time they have

been studied. These differences are sufficiently marked and constant to make it possible to distinguish the three strains studied as separate physiologic forms by cultural characteristics alone.

The cultural differences of the three forms of covered kernel smut from single chlamydospores when grown on carrot agar, potato-dextrose agar, and oatmeal agar are given in tables 1, 2, and 3, respectively. The differencens shown by cultures of the three forms growing on potato-dextrose agar are illustrated in figure 1. Color determinations were made

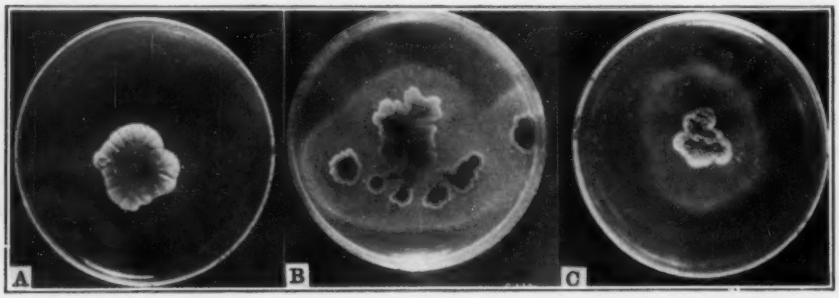

FIG. 1. Twelve-day cultures of three physiologic forms of *S. sorghi* grown on potato-dextrose agar at 80° F.
A. Physiologic form 1 from Pink kafir.
B. Physiologic form 2 from Dwarf Yellow milo.
C. Physiologic form 3 from feterita.

according to Ridgway's color standards and color nomenclature (7).

When grown on carrot agar the outstanding differences of these forms are as follows: Form 1 is characterized by an ivory-yellow color, and bacterioid consistency in young cultures. Later it becomes felted, the center usually being tufted in old cultures. Form 2 ranges from cinnamon-buff to cream-buff in color when young, turning to an umber color with age. The topography changes from beaded in young cultures to rather densely reticulate in fully developed cultures. Form 3 may be distinguished from the other two by its olive-buff color and its sparse spiny projections, which later disappear, the culture then taking on a beaded appearance.

When the cultures are grown on potato-dextrose agar, the colonies of form 1 have cream-buff centers with the remainder ivory-yellow. Their topography is relatively flat, either with or without reticulation. In form 2, the centers of the colonies are russet to cinnamon-brown and the remainder cream-buff in color, while their topography is rather flat with raised reticulations. In form 3, the colonies are pale olive-buff to dark olive-buff with very fine spiny projections and small beads.

When grown on oatmeal agar the outstanding color differences among these forms are as follows: Form 1 is cartridge-buff in the center and the

TABLE 1.—*Cultural characteristics of physiologic forms of Sphacelotheca sorghi on carrot agar. 12 days old*

Host	Form	Color	Topography	Surface	Consistency	Margin
Pink kafir (Bot. 1928 Row No. 23)	1	Ivory-yellow	Raised; center sparsely tufted; remainder reticulate	Waxy	Dry bacteroid	Lobate ridges; bacterioid
Milo (Bot. 1928 Row No. 43)	2	Cinnamon-buff to to Saccardo's umber	Raised; beaded to reticulate	do	do	Lobate; slightly beaded, bacterioid
Feterita (Bot. 1928 Row No. 21)	3	Olive-buff	Raised; portions of upright tufts; remainder beaded	do	do	Wavy; smooth with very fine striations, bacterioid

TABLE 2.—*Cultural characteristics of physiologic forms of Sphacelotheca sorghi on potato-dextrose agar. 12 days old*

Host	Form	Color	Topography	Surface	Consistency	Margin
Pink kafir (Bot. 1928 Row No. 23)	1	Cream-buff; margin ivory-yellow	Slightly raised; very finely reticulate	Waxy	Dry bacterioid	Wavy to lobate ridges, bacterioid
Milo (Bot. 1928 Row No. 43)	2	Center russet to cinnamon-brown; margin cream-buff	Relatively flat; raised reticulate ridges	do	do	Wavy, bacterioid
Feterita (Bot. 1928 Row No. 21)	3	Pale olive-buff	Slightly raised; beaded	do	do	Slightly wavy; mycelioid

TABLE 3.—*Cultural characteristics of physiologic forms of Sphacelotheca sorghi on oatmeal agar. 12 days old*

Host	Form	Color	Topography	Surface	Consistency	Margin
Pink kafir (Bot. 1928 Row No. 23)	1	Center cartridge-buff; remainder covered with a white felt	Flat	Center waxy; remainder dull	Center dry bacterioid; remainder mycelioid	Entire and mycelioid
Milo (Bot. 1928 Row No. 43)	2	Center russet; remainder white	do	do	do	Slightly wavy and mycelioid
Feterita (Bot. 1928 Row No. 21)	3	Olive-buff with spots of russet; margin white	Flat; raised beads	Waxy; margin dull	Dry bacterioid; margin mycelioid	Wavy, mycelioid

remainder is covered with a white felt. Form 2 is umber to russet in the center and white elsewhere. Form 3 is olive-buff with spots of russet, and a white margin.

The rate of growth of the three forms varies with the media on which they are grown. The rate of growth of all three forms on oatmeal agar is about the same, but on carrot and potato-dextrose agar form 2 grows more rapidly than either form 1 or 3. On carrot and potato-dextrose agar form 1 usually has been a more rapid grower than form 3.

The formation of wedge-shape or fan-shape sectors in single and multi-chlamydospore cultures of form 1 has been mentioned. These also have been observed in one of the cultures of form 2. These sectors differed from the parent cultures in one or more characters, such as rate of growth, consistency, topography, or color. Most of them differed by a single character, but a few differed in two or more. In all cases such sectors were sufficiently different from the remainder of the cultures to be easily noticeable. Transfers were made from the margins of the sectors and new cultures developed from them. These have been transferred frequently and have maintained their distinguishing characteristics without change (Fig. 2).

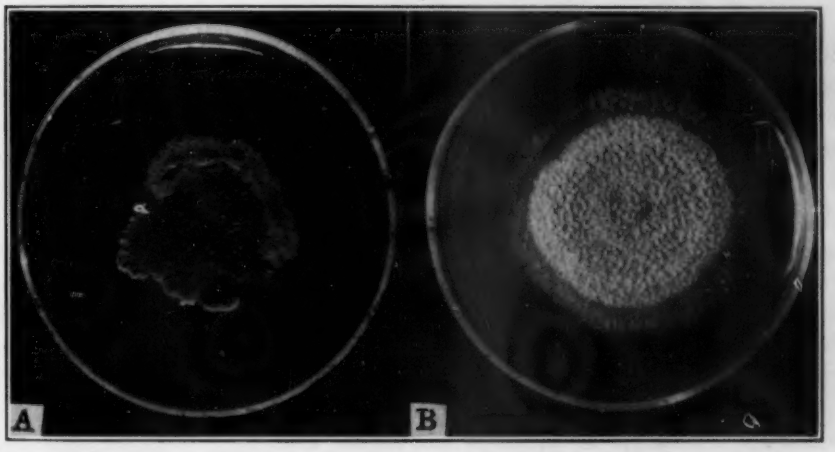

FIG. 2. Sectoring in physiologic form 2 of *S. sorghi* on carrot agar.
A. Main portion of culture bacterioid cinnamon-buff in color with a cream-buff margin. Sector mycelioid, gull-gray in color.
B. Culture grown from a marginal transfer from the sector shown in A.

Thus far only a limited number of monosporidial cultures have been made and no sectoring has been noted in them. In some cases two different types of cultures were obtained from separate monosporidial cultures of a single physiologic form. For example, bacterioid and mycelioid cultures were derived from monosporidial cultures of form 3. In all cases these cultures

were constant for their respective characteristics in subsequent generations.

Sectoring was of frequent occurrence in cultures of form 1, arising either from single or many chlamydospores. The phenomenon has not been observed in form 3 and only one case has been noted in form 2. Only a limited number of cultures has been studied thus far, however, and further studies may reveal them in all three physiologic forms. On the basis of the present experiments it seems that form 1 is relatively unstable, while forms 2 and 3 are very stable. This is of interest from a historical standpoint, as form 1 has long been known, whereas forms 2 and 3 have been observed only recently. Tisdale, Melchers, and Clemmer (11) discussed the possibility of hybridization of *S. sorghi* and *S. cruenta* (Kühn) Potter having given rise to a new form of sorghum smut. Leonian (5) has shown that many new types arise in Phytophthora by mutation, while Christensen (1) noted the same phenomenon for *Helminthosporium sativum* P., K., and B. Christensen and Stakman (2) and Stakman, Christensen, and Hanna (10) have demonstrated the formation of new forms of corn smut in culture by the same process. More recently Rodenhiser (8) noted mutations in *U. hordei* and *U. avenae* which differed from the parent cultures in color and rate of growth.

On the other hand, Stakman and Christensen (9) have shown that corn smut is heterothallic and that hyphal fusions of cultures of opposite sex were necessary before the fungus was able to fruit. Hanna (4) confirmed these observations for *U. zeae* and demonstrated the same phenomenon in *Sorosporium reilianum* Kühn (McAlp.). Dickinson (3) has also shown that cultures of different sex also frequently have strikingly different characteristics in culture.

Thus it seems that the sectoring noted in physiologic form 1 of *S. sorghi* may have been due either to mutation or to heterothallism. Further studies on these points are under way. The evidence at present points to heterothallism rather than to mutation, as the sectors observed arose in cultures from the diploid chlamydospores rather than the haploid sporidia. Although chlamydospores are uninucleate the single nucleus arises in the young spore cells by the fusion of two nuclei, according to Rawitscher (6). It should be possible, therefore, for sectors to arise by segregation or by the fusion of sporidia in the heterothallic culture.

SUMMARY

Three physiologic forms of covered kernel smut of sorghum are shown to be distinct in culture on various agar media. They differ in color, surface, consistency, margin, and rate of growth.

Physiologic form 1 attacks Pink kafir, but is unable to infect milo or feterita. Form 2 attacks milo to a moderate extent and Pink kafir severely,

but is unable to attack feterita. Form 3 attacks feterita to a moderate extent and Pink kafir rather severely, but is unable to attack milo.

Multi- and single-chlamydospore cultures were made of all three forms and monosporidial cultures also were studied to a limited extent. Sectoring was observed rather frequently in cultures of form 1 arising from chlamydospores, but not in monosporidial cultures. The other forms seemed to be very stable, sectors appearing only in a single instance in form 2 and not at all in form 3.

The cultural characteristics seemed very stable in most cases, successive transfers and new cultures from the original inoculum yielding cultures with the same distinguishing characteristics.

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SOME MICROSCOPICAL STUDIES ON PENICILLIUM DECAY OF CITRUS[1]

L. J. KLOTZ[2]

Histological observations were made on fresh and paraffined sections of fruit decayed by *Penicillium digitatum* (Fr.) Sacc. and *P. italicum* C. Wehmer. Pieces of lemon and orange fruit, including both the diseased areas and the healthy, noninvaded regions, were killed and fixed in chrom-acetic-urea solution, embedded in paraffin, sectioned 6 to 15 μ in thickness, and stained with Magdala Red and Licht Grün according to the method of B. T. Dickson (2) or the Pianeze method of Vaughan (11). The latter combines the stains malachite green, acid fuchsin, and Martius Gelb in one solution. With both methods the host tissue is stained green and the mycelium pink to red. I obtained better staining and differentiation with the former method.

Although the types of decay produced, respectively, by these two fungi are macroscopically easily differentiated [Fawcett and Lee (3), pp. 353–362, fig. 112], in these studies no microscopical differences were detected in the method of attack of the two organisms. The hyphae were without definite haustoria and were distinctly intracellular as well as intercellular [Fig. 1, A, B, and D and compare with Smith (10)]. Some hyphae had very apparent constrictions where they penetrated the cell walls. They were tortuous, ramifying, and extremely variable in thickness, ranging from 3 μ to 17 μ in our material, as shown in figure 1, B and E. The large, densely-granular mycelial threads completely filled some of the host cells (Fig. 1, B and D). The fungi penetrated all cells comprising the albedo and flavedo, including the cells of the oil glands, but avoided the space containing the oil. Hyphae of both fungi invaded the region of the juice vesicles, but in the material used, taken at the advancing margin of the rot, they were usually found only in the vesicle tissue next to the albedo, that is, in the cells of the stalks of the juice vesicles. Advanced stages of the decayed tissue were not studied by means of the paraffin method, but it was found that individual juice vesicles taken from fruit decayed by *P. italicum* always contained mycelium. The cells of the expanded part of the vesicle containing the juice seemed the last to be invaded.

Epidermis stripped from the margin of the decayed portion of an orange attacked by *P. italicum* showed that, in fruiting, the hyphae of

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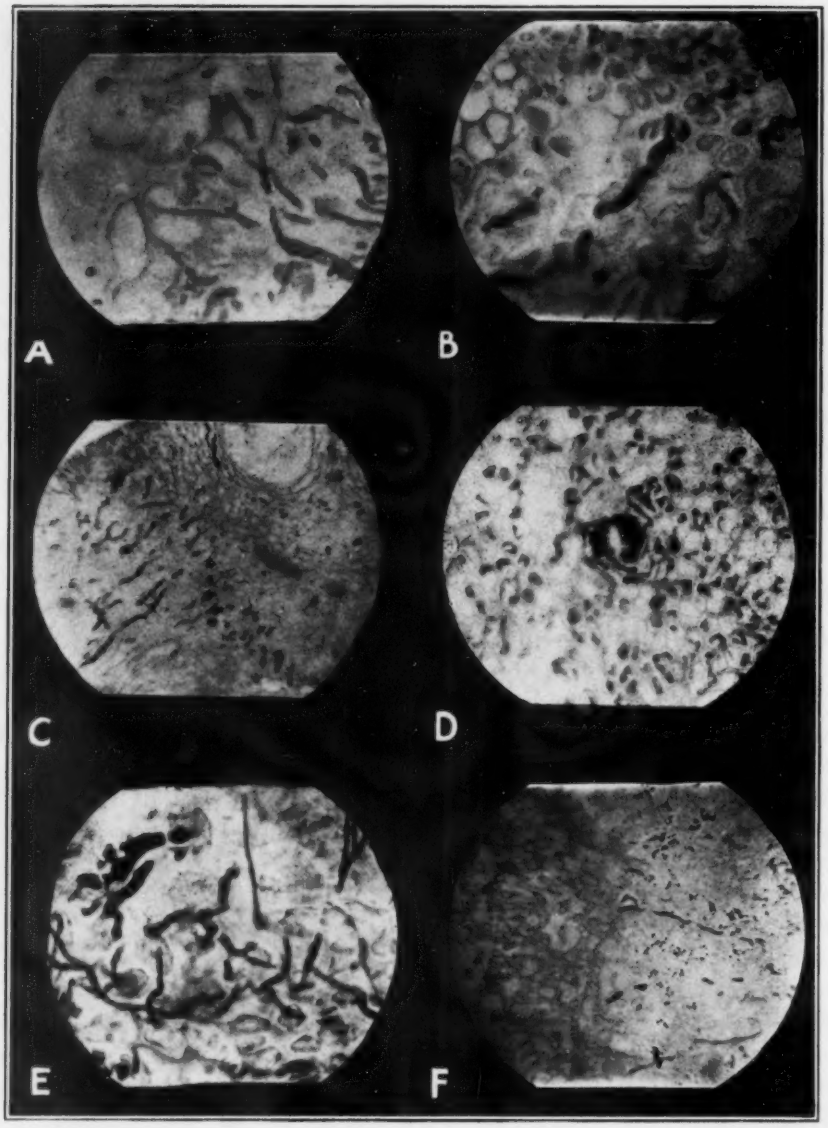

FIG. 1. Hyphae of Penicillia in rind of Citrus. A, B, C, E, F, in albedo. D, surface view of epidermis.

the rind collect substomatally as heavy-wall threads and crowd up through the surface, wedging and crowding apart the auxiliary cells, to form a fascicle of aerial hyphae upon which the condiophores and conidia ultimately appear (Fig. 1, D, and Fig. 2, A, B, and C). It is occasionally

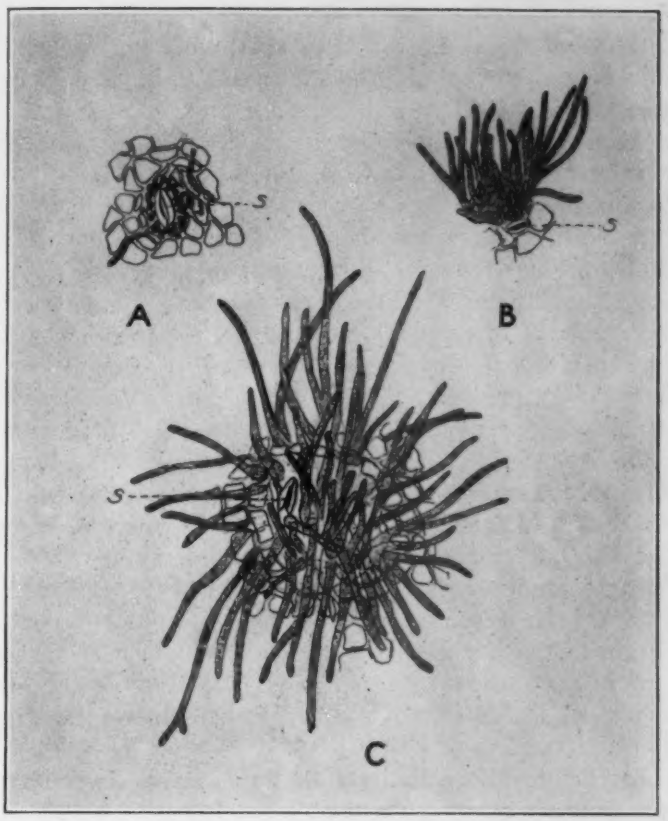

FIG. 2. Early stages of aerial fruiting hyphae on citrus. S—stoma.

possible to find the intact guard cells at the base of these fascicles. Sometimes the guard cells are crowded aside and are seen at the base of the periphery of the cluster of aerial hyphae (Fig. 2, B). In some instances the mycelial threads are seen penetrating the stomatal slit, but usually they surround the guard cells.

In these diseases little evidence was found of an affection of host tissue extending beyond the region actually invaded by the mycelium (Fig. 1, F). Johann (4, 5), working with a disease of corn seedlings caused by *Penicillium oxalicum*, found that the cells of the embryo and mesocotyl were apparently killed in advance of invasion by the mycelium The causal fungus, a weak pathogene and chiefly intercellular, excretes an abundance of oxalic acid. The author found that this acid, placed on the surface of the seedlings, produced lesions similar to those caused by the fungus. Yellowing and desiccation of the leaves, which are typical

symptoms of the disease, could be simulated by placing corn seedlings in dilute solutions of the acid. Nobécourt (6), on the other hand, believed that the toxins or enzymes of *P. glaucum* and *Rhizopus nigricans,* which disorganize plants, were intracellular, since there is nothing in a carrot-broth medium after the fungi have grown in it that attacks carrot; whereas, an extract of the macerated fungus mats possessed remarkable power to disorganize the plant tissue. It would seem, however, that if the cultures had aged there would have been sufficient autolysis of the old hyphae to have released some of the macerating principle. With the citrus decays it was found that the albedo parenchyma in the region occupied by the mycelium of *P. digitatum* and a short distance beyond, probably never more than 3 mm. (300 μ), shows maceration and collapse and takes a less dense stain with the Licht Grün (Fig. 1, F). This region was even smaller in the decay caused by *P. italicum,* the mycelium being not more than 2 or 3 cells away from the deeply-stained region. This may indicate an advance in action, but it is too limited to be determined easily by isolation methods. I failed repeatedly to isolate the organisms from solid tissue just beyond the margin of decay, nor were they found in fresh microscopic mounts made from this region. On the other hand, they were always present in the tissue that showed even the slightest softening. Complete collapse of the host cells was seen and finally only traces of them were found in the advanced stages of decay, the space being occupied by the mycelium of the fungi. These findings are in contrast to those of Rushton (8).

The above observations indicate that the macerating substances are for the most part intracellular, as Nobécourt found with *P. glaucum,* and are secreted in limited quantity only and near the tips of the living, invading hyphae. They probably are liberated *en masse* from old, dead, autolyzing mycelium in the regions of advanced decay. To test these observations the following simple experiments were made. Mycelial mats of the fungi were dried with acetone and ether, followed by a temperature of 45° C. for 24 hours; then ground to a fine powder and covered with toluene. The toluene was finally driven off by exposure to a temperature of 45° C. This material was mixed with twice its volume of water and inserted in cylindrical holes made in the albedo of orange and pummelo. The softening produced was distinct but limited to the margin of the holes, extending but a short distance into the albedo. A remarkable thing to be noted here was that some portions of the fungi, spores or resistant mycelium, were living after the drying process, the grinding, and the treatment with toluene, provided the toluene was not allowed to remain in contact with the powder but was at once exposed to a temperature of 45° C. and thus gradually driven off. Material which had been treated for a week with

toluene had no living fungus. Extracts of the fungus powder were made by placing a gram of the material in 100 ml. of distilled water for one hour, shaking vigorously at 5–10 minute intervals, and then filtering the suspension by suction through a double thickness of sterile filter-paper. Five-ml. quantities of the filtrate were injected hypodermically into the albedo of orange and pummelo. Observation at the end of 16 hours revealed marked softening of the treated albedo, which closely resembled the decay caused by the living fungi. The area of maceration was outlined with India ink and was observed as time passed to extend beyond this demarcation. Control fruit injected with distilled water was not macerated.

Injections of 1 per cent oxalic-acid solution caused a maceration in pummelo and orange rind, the latter being firmer and more turgid than the former. The portion of the rind affected by the acid was depressed uniformly, making a smooth, rather firm area. The effect produced by the extract of the dried fungus differed from that of the chemical in being softer and less uniformly sunken and of a faintly brown color. Moreover, the extract, unlike that of *P. oxalicum* studied by Johann (5), gave no qualitative test for the oxalate ion. Neither distilled water nor a 1 per cent solution of ammonia (C. P. 28 per cent) caused an effect on orange or grapefruit detectable in 18 hours. After 48 hours the orange injected with ammonia showed a slight browning and a shallow, irregular sinking; the pummelo continued to be unaffected at the end of that period. However, the rind of fruit injected with a 5 per cent solution of ammonia acquired within 5 hours a sayal-brown to cinnamon-colored area [Ridgway (7)], which was much firmer and considerably darker than that caused by the fungus extract. It has been observed by Savastano and Fawcett (9) that oranges decayed by Penicillium species at high temperatures acquire a discoloration resembling that caused by the ammonia. The resemblance of ammonia injury to Phomopsis stem-end rot had been recorded by Bahgat (1). It is possible also that in the Penicillium decay the ammonia liberated by the desaminases and desamidases of the fungi is in part responsible for the injury.

SUMMARY

To sum up, it was found, among other things, that *Penicillium digitatum* and *P. italicum,* important organisms in the decay of Citrus fruits, grow inter- and intracellularly in the albedo parenchyma, flavedo, and epidermis and finally invade the juice vesicles.

The hyphae of these fungi, which are very variable in width, collect substomatally and thrust aerial fruiting hyphae through the epidermis in the neighborhood of the auxiliary and guard cells. The mycelium exhibits but a very limited effect in advance of invasion.

The nature of the decay by the living fungus was compared and contrasted with the softening caused by injections into the albedo of solutions of oxalic acid and ammonia and of an extract of the fungi.

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FURTHER STUDIES OF PRIVET ANTHRACNOSE

A. J. Mix

DISTRIBUTION OF THE DISEASE

In an earlier paper (4) on privet anthracnose, caused by *Glomerella cingulata* (Stonem.) Spaulding and von Schrenk, which included an account of the history and distribution of the disease, it was stated that, although the fungus *G. cingulata* had been the subject of much investigation, there seemed to have been no mention of privet anthracnose in the literature later than the account given by Atkinson (2) in 1892. This statement was due to the fact that a reference by Clinton (3) had been overlooked. Clinton records the occurrence of the disease in Connecticut in 1913 and also mentions the occurrence of basal cankers and the consequent death of the plant.

Privet anthracnose seems to be more widely distributed than the references cited above would indicate. Anderson and others (1), in their Check List of Diseases of Economic Plants in the United States, mention this disease as "widespread east of the Great Plains." Its destructive occurrence within the Great Plains area has been previously reported (4).

OCCURRENCE OF THE DISEASE ON A NEW PRIVET

In all of the above-mentioned accounts of privet anthracnose, the host species concerned has been *Ligustrum vulgare* L. In the fall of 1925, a letter was received from Dr. H. W. Anderson describing the occurrence of the anthracnose in an Illinois nursery. It had caused severe loss in some plantings of a new privet called Lodense. Some Lodense privet plants which were received from a nursery in 1927 and planted on the campus of the University of Kansas developed in the summer of 1929 typical twig blight and cankers of the anthracnose. *Glomerella cingulata* was isolated from these blighted twigs and cankers. Beyond these instances, nothing further is known of the occurrence of the anthracnose on Lodense privet, nor, for that matter, of the extent to which the Lodense privet has come into cultivation.

THE LODENSE PRIVET

The Lodense privet was introduced to the trade by the Henry Kohankie and Son Ornamental Nurseries, Painesville, Ohio. Mr. H. J. Kohankie states that they still have the original plant and that it was found among a lot of seedlings of European privet which they imported from France some years ago. It is his opinion that it is a "sport" of *Ligustrum vulgare*.

It has not been possible to determine the botanical relationships of this privet. It is not recognized in botanical literature under the name Lodense. In the general appearance of its foliage, flowers, and fruit it resembles *L. vulgare,* but in comparison with that species possesses a decidedly compact, dwarf habit. Miss Amelia Babcock, in the course of some comparative morphological and anatomical studies of species of *Ligustrum,* has found the Lodense privet to differ as widely from *L. vulgare* as did several other recognized and named species. On the basis of her work it seems undoubtedly distinct from *L. vulgare, L. ibota, L. amurense, L. ovalifolium, L. ibolium, L. quihoui,* and *L. regelianum.* (Miss Babcock's work is reported in an unpublished thesis of the University of Kansas.)

PURPOSE OF EXPERIMENTS

The inoculation experiments, to be described below, were undertaken with two purposes in mind. The first was to test the susceptibility of the Lodense privet to anthracnose. This seemed worth while since the inoculation experiments reported earlier (4) were performed with several species of privet, of which only *Ligustrum vulgare* proved susceptible.

The second purpose was to determine whether *Glomerella cingulata* from apple could attack privet, and vice versa. Shear and Wood (6) have shown that this fungus is morphologically and physiologically the same on many different hosts, but among the large number of cross-inoculation experiments which they performed they did not include any between privet and apple. Since susceptible privets may be planted in localities where apples are grown and where bitter rot occurs, it seemed worth while to determine whether the fungus can pass from the one host to the other. In the earlier inoculation work (4) it was learned that *G. cingulata* from privet could cause decay in ripe apple fruits. A few successful inoculations of privet branches with *G. cingulata* from apple fruit were also reported. The inoculations described below were intended to secure additional data on this latter point.

INOCULATIONS

Plants of Lodense and of European privet were secured in the spring of 1927. These appeared to be about two years old. Some of them were planted in the greenhouse and a few out of doors. Additional plants were secured by making cuttings from the branches of the older plants. Inoculations were performed on the older plants immediately and on the younger plants during their first season of growth. Since there proved to be no difference in susceptibility between plants of different ages, except that the younger plants died more quickly, nor between plants growing in the greenhouse and out of doors, the results have been summarized

without regard to age or location of the inoculated plants. These results are recorded in tables 1, 2, and 3.

TABLE 1.—*Results of inoculating privet plants with Glomerella cingulata by means of incisions into main stem below ground*

	Kind of privet	Source of culture	Number of incisions	Number of infections
Inoculated	Vulgare	Privet	20	19
"	"	Apple	28	4
Controls	"	.....	7	0
Inoculated	Lodense	Privet	7	6
"	"	Apple	6	4
Controls	"	.....	4	0

TABLE 2.—*Results of inoculating privet plants with Glomerella cingulata by means of incisions into branches*

	Kind of privet	Source of culture	Number of incisions	Number of infections
Inoculated	Vulgare	Privet	10	10
"	"	Apple	13	1
Controls	"	.....	11	0
Inoculated	Lodense	Privet	3	3
"	"	Apple	11	10
Controls	"	.....	11	0

TABLE 3.—*Results of inoculating privet plants with Glomerella cingulata by spraying with spore suspensions*

	Kind of privet	Source of culture	Number of plants	Number of plants becoming infected
Inoculated	Vulgare	Privet	10	10
"	"	Apple	3	0
Controls	"	.....	3	0
Inoculated	Lodense	Privet	2	2
"	"	Apple	8	3
Controls	"	.....	3	0

Two methods of inoculation were used: 1. Incisions into bark and wood (Tables 1 and 2), with insertion of spores and mycelium from culture, the wounds being left unprotected; 2. Spraying of foliage and stems with sterilized-water suspensions of conidia from culture (Table 3), the plants being subsequently covered with bell jars for 48 hours. Controls consisted

in uninoculated and unprotected incisions and in spraying plants with sterilized water and covering them as above.

Two cultures of *Glomerella cingulata* which had been isolated from European privet were used for these inoculations, and three cultures from apple. One of these apple cultures had been isolated from fruit by Dr. H. W. Anderson, and the other two were isolated from Willow Twig apple fruits affected with bitter rot, also kindly furnished by Dr. Anderson. Since no significant differences in pathogenicity seemed to exist between different cultures from the same host, the culture numbers are not included in the tables.

Infection was considered to have occurred when an incision was found unhealed and a considerable portion of the wood of the stem was discolored and dead, or, in the case of spray inoculations, when definite leaf spots and stem cankers (always a considerable number on one plant) had developed. *Glomerella cingulata* was recovered by reisolation from a number of inoculated Lodense and European plants.

CONCLUSIONS FROM INOCULATIONS

A study of the results shown in these tables seems to justify the following conclusions:

1. Lodense privet appears fully as susceptible to infection by *Glomerella cingulata* as does European privet and it should not, therefore, be recommended for planting as a hedge plant in localities where the fungus occurs. It may be emphasized in this connection that for use in ornamental hedges a plant needs to be highly resistant, if not entirely immune from disease.

2. *Glomerella cingulata* from apple is capable of causing infection on Lodense and European privet, even if somewhat less readily than the same fungus from privet. A locality in which apple bitter rot occurs would therefore be unsafe for these privets.

It may be further noted that Lodense privet seems to be more readily attacked by *G. cingulata* from apple than is European privet.

INOCULATIONS OF APPLE BRANCHES

Inoculations of apple branches with *Glomerella cingulata* previously reported (4) resulted in no infections. A new series of such inoculations with cultures of *G. cingulata* from both privet and apple was made in 1927. These were wound inoculations made by means of incisions into one-year old and two-year old branches of young Ben Davis, Jonathan, Northwestern, and seedling apple trees. From 20 to 30 such inoculations were made with each variety. No infection resulted.

Von Schrenk and Spaulding (5) are apparently the only investigators who have successfully inoculated apple branches with *Glomerella cingulata*.

In their experiments the wounds were covered with grafting wax after inoculation, while in those reported here the wounds were left unprotected. This may account for the difference in results.

SUMMARY

Anthracnose, caused by *Glomerella cingulata* (Stonem.) Spaulding and von Schrenk, besides being widely distributed in the United States on European privet, occurs also on a new privet called Lodense.

The botanical classification of the Lodense privet is not known, but it seems to be distinct from *Ligustrum vulgare* and from several of the other commonly grown species of privet.

Inoculation experiments have shown that *Glomerella cingulata* from privet and from apple can cause infection on both European and Lodense privet.

A series of inoculations of the young branches of apple trees with cultures of *G. cingulata* from both privet and apple resulted in no infection.

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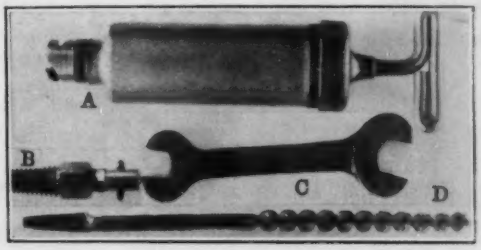
A SIMPLE TREE INJECTOR

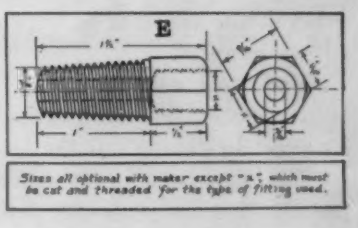
RUSH P. MARSHALL

Interest has been shown in a simple method of tree injection demonstrated by the writer at a conference of shade tree workers held in Stamford, Connecticut, in the summer of 1927. Believing that pathologists who work with tree species will find the method useful for rapid injection of small quantities of material, the apparatus used is here briefly described.

It consists in the application of the automobile grease gun to the injection of trees. With the exception of a single fitting, the parts may be borrowed from the automobile and laboratory tool box or purchased at small cost from the nearest hardware store.

This particular fitting (Fig. 1, B and E) can be turned out in a few





minutes by a machinist if the experimenter himself has not the facilities for making it. It is a brass tube threaded at one end to receive an Alemite bearing and cut at the other to form a wood screw. A working drawing is supplied. The dimensions given are those of the fitting used by the writer, but they are optional.

A number of guns of the regular and "Zerk" types were experimented with. Of those tested, the small, regulation, Alemite gun, sold for use with 600 W. oil, was found most suitable for the present purpose. The complete outfit, with the exception of a brace, is easily carried in the pocket (Fig. 1; A, B, C, and D); it has no hose to develop leaking connections; it is said to give a pressure of 500 pounds to the square inch; it will hold two ounces of material.

These guns are designed for use with heavy oil or light grease, and no difficulty is experienced in injecting trees with substances which approximate their consistency. The apparatus is less practical for the injection of water solutions, and, if they are to be used, care is required. The leather plunger must be in good order and neither worn nor folded. If new, it

should be softened with some such dressing as neatsfoot oil or Viscol and then coated with cup grease. When larger guns with hoses are used, it is generally necessary to pack the hose connections with spray-pump packing to prevent leakage. Guns of the larger type inject heavy materials readily, but are practically worthless for aqueous solutions.

The process of injecting the tree is simple. Using a brace with a bit the same size as the small end of the wood screw, a hole is bored nearly through the trunk. The mouth of the opening is then faced with a countersink, and the wood screw of the fitting into which the Alemite bearing has previously been screwed is turned into the wood with the help of a wrench. The remainder of the operation consists in turning down the handle as in greasing a car, except that in using water solutions it is necessary to make the first several turns very quickly in order to develop sufficient pressure to create good contact between the nozzle of the gun and the bearing. The rapidity with which the material may be forced into the wood varies greatly with the tree, the substance used, and the time of application. In general, the contents of a two-ounce gun can be injected in from five to fifteen minutes.

Following use, the gun is easily cleaned. It may be sterilized with boiling water, but sterilization by dry heat is to be avoided on account of its effect in drying out the leather plunger.

The apparatus here described has been used in attempts to force preservative materials into pruning wounds, to inoculate trees with fungous cultures, and to make experimental injections to control borers. It has worked effectively for substances the consistency of which is approximately that of grease or heavy oil, and poorly for aqueous solutions. Since the rapidity with which the material is forced into the trunk under high pressure does not give time for any great degree of distribution during the process of injection, the amount of material which can be forced in is limited. For this reason, the grease gun cannot supplant injection apparatus in ordinary use. It is valuable where the rapid injection of not more than several ounces of material to a hole is involved and it will inject many materials of a consistency too thick to permit of injection by usual methods.

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PHYTOPATHOLOGICAL NOTES

A blight of flowering almond, Prunus glandulosa Thunb.—Diseased specimens of the dwarf flowering cherry or flowering almond, *Prunus glandulosa* Thunb., have been observed at Lawrence, Kansas, during the past three seasons. In each of these years the disease was evident in late spring and early summer, following a rather prolonged period of wet weather. Blossoms, leaves, and twigs turned brown and died and were frequently covered with the conidial tufts of a Monilia. In some cases branch cankers developed around the bases of affected twigs.

From such blighted leaves and twigs a fungus was readily isolated. It proved to be the Monilia stage of the common brown-rot fungus, *Sclerotinia fructicola* (Wint.) Rehm. In cultural characters and in morphology of mycelium and conidia formed in culture it appeared closely similar to, or identical with, strains of the same fungus isolated from cherry and apple fruits. Sound fruits of peach, plum, and apple developed typical brown rot when inoculated with the Monilia from cherry, apple, and flowering almond. Shoots of flowering almond with opening blossoms were removed, sprayed with spore suspensions prepared from cultures of the three strains mentioned above, and kept, with their lower ends in water, under bell-jars lined with moist filter-paper. Blossom and leaf blight resulted in all cases. The fungus was reisolated from each set of twigs.

So far as can be determined, the disease has been reported previously only once. W. C. Sturgis (Connecticut Agr. Exp. Sta. Rept. 1898: 261–267. 1899), in describing an outbreak of brown-rot blossoms and twig blight in Connecticut in 1898, states: "The trouble . . . was very common also on the ornamental shrub known as the Double Flowering Almond." I collected specimens of the disease (from which the fungus was later isolated) at Collinsville, Connecticut, in the summer of 1928.

The disease does not appear to be very destructive in this locality. It has been kept in check during the past three seasons by careful pruning out of affected branches, and such pruning has left the shrubs in thrifty condition.—A. J. Mix, University of Kansas, Lawrence, Kans.

Brown-rot leaf and twig blight following peach-leaf curl.—The usual account of the peach-leaf-curl disease states that the curled leaves eventually die and fall from the tree. In reporting (Mix, Phytopath. 14: 217–233. 1924) some studies of *Exoascus deformans,* I called attention to the fact that, after ascospore dispersal has occurred, curled leaves are commonly invaded by other fungi. These fungi accomplish the final destruction of the leaf, but have never been observed to pass from the curled portion of the leaf into the adjacent healthy portion. For this reason no particular

attention has been paid to their identity, but species of Alternaria and Fusarium have been observed and a species of Cladosporium is common.

In June, 1928, while collecting peach-leaf curl in unsprayed orchards in the vicinity of New Haven, Connecticut, I noticed that nearly all of the curled leaves and shoots were being destroyed by the brown-rot fungus, *Sclerotinia fructicola* (Wint.) Rehm. In contrast to the fungi mentioned above, this fungus was growing from the curled portions of leaves and twigs into the adjacent healthy portions. In some cases healthy leaves in contact with curled leaves were being invaded by the brown-rot fungus. In short, brown-rot leaf and twig blight were abundant in these orchards and were at this time confined exclusively to leaves and twigs previously infected by the leaf-curl fungus. Peach fruits were apparently too young to be susceptible to brown rot; at any rate, no diseased fruits were found.

It was clear that in this season and in this locality the establishment of the brown-rot fungus in peach orchards had been aided by the previous establishment of the curl fungus. It is not known how common such an occurrence may be. It has not been observed under the less humid conditions of eastern Kansas. It seems, however, that here is an added reason for spraying peach trees to prevent peach-leaf curl.—A. J. MIX, University of Kansas, Lawrence, Kans.